

26 June 1980

What not to say about interferon

PHYSICIANS on both sides of the Atlantic appear to be worried at the lively public interest in the possibility that interferon may at some stage have a part to play in the treatment of cancer. Two weeks ago, for example, the Glasgow Health Board appealed for what sounded very much like a moratorium on publicity about the clinical trials being carried out with the meagre quantities of interferon now available. Last week, a number of distinguished physicians in the United States made a very similar appeal. Everybody will sympathize with the dilemma of those working in the treatment of cancer when they are confronted by patients and their relatives pleading for the better chance of survival which they are frequently persuaded interferon might provide. The physicians are not concerned to shuffle off their professional responsibilities; rather, they are anxious to protect their patients and their relatives from such needless distress as there is bound to be when it is believed that a potentially beneficial drug is being withheld. It is no comfort to those concerned to know that the quantities of interferon now available are sufficient only to sustain a handful of clinical trials here and there, or that they are excluded by the protocols laid down from such clinical trials as are being conducted. But alas, a moratorium on publicity will not suffice. The need is rather for more but better publicity.

For the truth is that the professional community can no longer hope to conceal from the wider public its own motives and excitements. That this should have become the case is not a misfortune but the opposite — a sign that after many decades in which the professional community has wrung its hands at the poverty of the public understanding of what science is about, the point may now have been reached at which people in general are beginning to appreciate what is happening in the laboratories. Who would turn that clock back? Moreover, it can take very little ingenuity for the general public to discover that many professional people are indeed excited about interferon because of the possibility that it may have a role to play in the treatment of at least some kinds of cancer. Thus a correspondent writing in last week's *Nature* about the structure of a human fibroblast interferon gene, and about the structural relationship between leukocyte and fibroblast interferons, explained in passing that part of the interest in these two molecules stemmed from "their potential as *in vivo* anti-viral and anti-tumour agents". So it does, and it would be dishonest even of simple biochemists (if there are such) to pretend otherwise. The questions for the professional community, physicians included, is therefore not that of avoiding publicity but of making sure that the harmful consequences of publicity are avoided.

How should that be accomplished? The short answer, paradoxically, is to say more, not less. Perhaps the most urgent need is for a wider understanding of the reasons why the pace of research with interferon has recently so rapidly accelerated. After all, there will be many who recall that the first mention of interferon dates back for more than twenty years, when in Britain the Medical Research Council of the day held a widely reported press conference to announce the discovery of what seemed to be a universal anti-viral agent. What, people will rightly ask, has been happening in the interval? The answer is, of course, instructive. After the initial excitement, it quickly emerged that the problems of isolating interferon in any but the smallest quantities were such that people would quickly have become disheartened at the prospect of setting up industrial production lines to make interferon. In any case, several years went by in

which people almost lost their patience in their attempts to characterize the material properly (which is not surprising in the light of the more recent discoveries of several different but related types of interferon, apparently produced by different genes). In reality, it is only within the past decade that techniques have been worked out for setting up and stimulating cell cultures that will yield measurable quantities of pure interferon. It is remarkable how much the techniques of molecular biology — nucleic acid hybridization and the like — have contributed already to the characterization and purification of interferon. It goes without saying that there must be great excitement at the possibility that genetic manipulation may be applicable to the production of interferon on a commercial scale — a prospect that remains to be substantiated.

Thus even as things are, interferon is not a drug in the sense in which that word applies to aspirin or cyclophosphamide. It remains a name for a group of materials, only some of which have been fully characterized and none of which can be manufactured in the ordinary sense of that word. Moreover, the natural role of interferon in or outside the cells in which it is produced remains obscure. What has emerged, after more than twenty years, is that interferon plays a part still to be fully defined in the regulation of viral replication within eukaryotic cells, and in some way yet to be defined, with cell replication as a whole. The possibility that interferon may be an anti-tumour agent springs more from clinical studies than theoretical understanding. By the same test, however, interferon used as a drug must be expected to have side-effects — even though there may be theoretical reasons for hoping that these may turn out in some cases to be less damaging than those which accompany (and sometimes limit) some of the cancer therapies now in use.

No physician would of course be rash enough to base his treatment of real patients on what he or she would no doubt call fairy stories such as these. Physicians are right to ask that the hints and nudges provided by laboratory experiments should not guide the design of methods of treatment until their efficacy and their safety have been demonstrated by means of clinical trials. (Increasingly, patients and potential patients insist on nothing less.) And there is a host of practical questions to be answered — what doses should physicians think of using against which kinds of cancers, what precisely are the side-effects and how might they be mitigated? Even if the supply of interferon were not limited (as it will be for some time to come), and even if the highest hopes for interferon are borne out by the trials now under way — and they may not be — it would be some time, say a couple of years, before interferon could be widely used in hospitals. As things are, the time-lag is likely to be longer before interferon could be made generally available. That is another essential element in what needs to be widely understood.

But how does one set about telling a patient suffering from cancer that there may at some stage be a more effective treatment than at present, but that it cannot be generally available for some years, by which time he or she may be dead? Nobody will envy physicians such tasks. It is however in the public interest that they should be tackled, and tackled energetically. In other less spectacular fields of pharmaceutical innovation, the lack of a general appreciation of what is involved in the development of new drugs is a serious brake on the pace of innovation. The steps now being taken to tell whether interferon has any substantial therapeutic value are a splendid illustration of what must be done



to bring a new drug into therapeutic use. The very fact that interferon has become such a powerful focus of public interest provides the professional community with a valuable opportunity for public enlightenment.

To be sure, the enlightenment will not be comfortable news for all those who receive it. Some will learn that they may be denied the chance to benefit from some innovation. It is however entirely consistent with the temper of the times that people should have to learn to live with the painful knowledge that what happens to them, literally their fate, may be intrinsically determined by chance. Curiously enough, people were more ready to accept this hard truth in mediaeval times, no doubt because of their common conviction that heaven was, in any case, a better place. Now that circumstances have changed, more robust philosophies are needed. It would be unfair to ask that physicians alone should shoulder the whole burden of helping their patients to come to terms with unlucky chance — but it would be wrong of them, and a disservice to their craft, if they were to fudge the issue.

As it happens, recent professional practice leaves very little to be asked for. The Medical Research Council, commenting last week

on the report by Dr David Sacher and Professor D.C. Burke (*Nature*, 12 June) that it has been possible to prepare monoclonal antibodies against human interferon which may be of service in the preparation of pure material, did not flinch from using the phrase “possible anti-tumour activity” but went on to refer to the urgent need “to test whether interferon really is useful in the treatment of any or all cancers”. The Imperial Cancer Research Fund, which announced last week the allocation of £1 million to a clinical trial with interferon in the next two years, made very similar noises. In the past few weeks, most newspapers have similarly behaved sensibly and properly, mentioning the “possible anti-tumour activity” of interferon only with a warning that the case has not been established. That is how it should be. In this day and age, it would be ridiculous to conceal the promise simply because the promise has not yet been substantiated, just as it would be irresponsible to pretend that interferon has already been shown to be beneficial. It is also essential that everybody should understand that interferon may yet turn out to have no role in the treatment of cancer of any kind. Or it may simply be another anti-tumour agent.

Can DNA properly be called selfish?

The response to the articles which appeared earlier this year on the theme of “selfish DNA” (Doolittle and Sapienza, Orgel and Crick, *Nature*, 17 April) has understandably been voluminous; the notion is, after all, mildly shocking. In essence, the authors are concerned to account for the presence in eukaryotic cells of much DNA that appears to have no function — the so-called “junk” DNA. They note that DNA sequences — any DNA sequences — are above all capable of replication; that the environment of the cell nucleus is well-suited to such replication; and that once a DNA sequence has been incorporated into the eukaryotic genome, there is no particular mechanism by means of which it can be got rid of. So, the argument goes, if there are DNA sequences which, because of their structure, are incorporated into the genome more easily than other sequences, they will tend not merely to stay there but to multiply, at least until the metabolic burden of replicating all this functionless DNA at every cell division becomes too great. This is selfish DNA — and the batch of comments on the subject which appears on pages 617–620, mostly approving of the notion as a stimulating idea, shows that not everybody accepts that something like this goes on in molecular evolution. Indeed, both the comments now published, and the general gossip about selfish DNA that there has been, suggest that people are uneasy about the concept.

Part of the trouble is undoubtedly semantic. Implicitly at least, people appear to be asking (among other questions) whether, and if so in what circumstances, it can be proper to attribute to a class of molecules the property of selfishness. For are we not told, from a quite tender age, that we should avoid anthropomorphism like the plague? And while it may be proper to extend the notion of selfishness, originally intended as an attribute of individual human beings that distinguished them from others, to animate species as a whole, is it not misleading to call a class of mere molecules “selfish”? Certainly the usage goes against the grain. No doubt if somebody could think of a better word, everybody would use it. Yet the image is defensible.

First, if it is at all acceptable that the word “selfish” should be applied to the behaviour of a species in, say, its competition with another, who is to say that it should not also apply to the competition of one species of DNA molecule with another? Nobody, of course, pretends that DNA molecules are in themselves living things; in present circumstances, DNA molecules cannot replicate unless they are made up into intact organisms of some kind, virus particles at the simplest. But it would be excessively pedantic to ignore the sources of the concept now advanced, and in particular the way in which it has emerged from the studies intended to throw light on the origin of life (in which Orgel is one of the outstanding exponents), and which have

plausibly suggested that in the *primaeva* soup, as it is called, the course of molecular evolution may have been determined by competition between different species of RNA molecules. The authors of the concept of selfish DNA might fairly ask how, in such circumstances, the concept of life as such is to be defined? And if the word “selfish” can be applied to a contemporary species in competitive evolution with others, why should it not also be applied to molecules in competitive molecular evolution? If there is an error, it is in the use of “selfish” in any but its original meaning.

The second justification of the use of “selfish” applied to DNA is the prior use of the word in relation to a gene. Thus Dawkins, in 1976, used the title “The Selfish Gene” for his account of how it appears that the effect of natural selection is to favour favourable genes and not strictly the organisms which carry them. This is, of course, a contentious field. The familiar aphorism that “the organism is DNA’s way of making more DNA” may turn out to be more than a mere joke, but whole-organism biologists find it offensive. In the present argument about selfish DNA, however, the use of “selfish” is justified on literary grounds: in a field in which many people are familiar with at least the title of Dawkins’s book, to apply the same word to DNA suggests graphically what the authors are driving at. Is that not how the language itself evolves?

None of this constitutes a licence for the use of anthropomorphic words wherever and whenever they have some suggestive or figurative value. It is, indeed, to be hoped that the word “selfish” will wither away once people have grasped the point that Doolittle and Sapienza and Crick and Orgel are trying to make. On present form, that may take some time. The question of whether they are right or wrong is clouded by other semantic issues. Is it, for example, proper to use the term “natural selection” to refer to the preferential emergence of some species of nucleic acid molecule either in the *primaeva* soup or in the environment of the eukaryotic nucleus? Part of the trouble is that natural selection is synonymous with Darwinian selection but selfish DNA has Lamarckian tendencies. Unfortunately the neutral word “selection” is not a sufficient substitute, implying as it does that the selection is undertaken by some unspecified agent; “to be selected”, on the other hand, is clumsy and only strengthens the habit of writing in the passive. This, however, is a minor issue compared with the way in which “selfish” appears to have provoked in many people’s minds, almost by free association, the belief that the selfish DNA must be the antonym of “altruistic DNA” and that the questions now raised are somehow linked with those which keep sociobiologists busy at public speaking. That is a cruel misfortune.

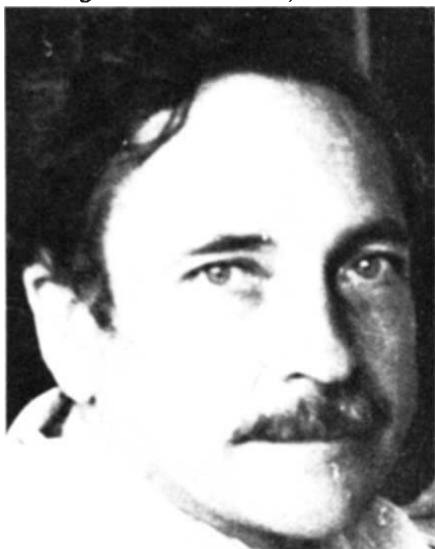
Nairobi centre's director to quit

THE World Bank's network of international agricultural research centres has been dealt a serious blow with the departure of Dr A C Allison as director of the International Laboratory for Research on Animal Diseases in Nairobi.

Dr Allison, a distinguished immunologist, was appointed director at Nairobi just over a year ago. He said on the telephone from Nairobi last week that the decision that he should leave his post was taken at the meeting of the governing council in April. Dr Allison says that he intends to stay in Nairobi until the review of the laboratory's five-year plan is completed in October, and possibly until the next council meeting in the spring of 1981.

Allison is the third director of ILRAD to leave in the six short years of its existence. The first died and the second, like Allison, resigned. It is an open secret that Allison and some members of the scientific and administrative staff at Nairobi have been at loggerheads almost since the beginning. Last week, Allison said that he had not been given the support he had reason to expect from the council itself.

ILRAD is one of the more recently created of the international centres now run by the Consultative Group on International Agricultural Research, a consortium



Ex-director designate?

of donor organizations among which the World Bank is the chief contributor. The founder members of the network of nine centres were the wheat and rice research centres in Mexico and the Philippines respectively.

Unlike other centres in the CGIAR network, ILRAD was set up to carry out a somewhat specific task — the protection of animals against trypanosomiasis and theileriosis (or East Coast Fever), a tick-borne disease fatal to large proportions of susceptible cattle.

ILRAD's first big success came soon after the foundation of the laboratory in

1974, when a technique for the *in vitro* culture of trypanosomes was developed. Since then it has seemed that the development of a vaccine against trypanosomiasis, African sleeping sickness, may be complicated by the antigenic variability of the infective organisms as recovered from infected animals.

Still more recently, however, it has appeared that genetic variability is by no means as apparent in the tsetse fly vectors of the disease, which appears to suggest ways in which the disease may be tackled. The prospects of vaccines against theileriosis are, by contrast, bright — some say that another two years may be sufficient.

Short commons for nuclear physics

Washington

ONE of the most disruptive effects of the budget battles currently being fought in Washington has been the defeat of several attempts to map out medium-term strategies for the support of basic research. A prominent victim is nuclear physics.

Earlier this year, physicists advising the Department of Energy and the National Science Foundation had proposed — and the Administration had accepted — that stability should be brought to nuclear physics funding by planning for a constant real growth of about three per cent over the next five years (just as three years ago agreement was reached to keep funding level in real terms for high-energy physics).

In particular, about \$20 million a year would be allocated for the construction or upgrading of research facilities. This would be about one-sixth of the total, and a considerable increase on the average equivalent of \$1.3 million a year spent over the past two decades.

A list of priorities was agreed with the department and was headed by the superconducting cyclotron now under construction at Michigan State University and a tandem linac accelerator system (Atlas) at the Argonne National Laboratory, for which funds had already been requested in the 1981 budget. Following these were plans for an electrostatic accelerator at Washington University and the upgrading of a tandem accelerator at Yale, with other projects climaxing in a national continuous beam high-energy electron accelerator.

Now the whole scheme is threatening to collapse like a house of cards. In revising its budget request in March, the Administration decided that construction at Atlas should be deferred. And last week the House of Representatives Appropriations Committee, taking a broad swipe at the whole energy research budget, recommended that rather than increase the Department of Energy's budget for nuclear

The laboratory has a permanent staff of some 40 scientists, together with students and visiting fellows. The annual budget is now approaching \$10 million which has provoked some criticism of the laboratory on the grounds of extravagance. In practice, the costs are not much out of line with those of other laboratories in the World Bank network, and are held to reflect the costs of supporting a largely expatriate staff in developing countries such as Kenya.

The chief anxiety of the laboratory management now appears to be the problem of recruiting their fourth director in six years in circumstances that may appear to potential candidates to be unpropitious.

physics by seven per cent as had originally been proposed, it should be reduced by five per cent below its current 1980 level in 1981, in real terms a contraction of about 15 per cent. Operating funds would fall to their lowest level since the early 1960s, and construction costs would be cut from \$13.3 million to \$9.3 million by delaying construction of the Michigan machine.

Before the latest cuts, nuclear physicists had been optimistic that they were seeing the end of a decade's decline. Central to this optimism had been the DoE's apparent acceptance of a report prepared by its Nuclear Science Advisory Committee, chaired by Professor Herman Feshbach of the Massachusetts Institute of Technology.

The report sets out a detailed rationale for a slow but steady increase in funding over the next five years. Particular attention is paid to the need for increased funding for new medium-sized and large facilities, as well as the upgrading of existing facilities. But the report also emphasizes that these should not be funded unless "a clear plan of action is developed that shows that the required balance in (national) capability can be retained".

In a normal budget year, this is the type of language that appeals to congressional committees. This year, it has cut little ice, and the promises of the January budget request seem unlikely to materialize.

At the National Science Foundation, for example, the Administration had suggested that the physics budget should be increased by 16 per cent to compensate for the relative growth of the biological sciences during the 1970s. This figure has been successively cut back, and is unlikely to end up as more than an eight or nine per cent increase.

It is the DoE's budget, however, which is threatened with the most damage. Here the House Appropriations Committee, shifting funds into politically popular dam construction projects, has proposed that the whole of the energy research budget should be kept at roughly the 1980 level, a

real contraction of about ten per cent.

The cuts would be shared across the board. The high-energy physics budget, for example, would have a proposed growth of 11 per cent cut to three per cent, with a \$20 million cut in operating funds for the major accelerator laboratories and in particular a \$5 million cut in funds for the construction of Fermilab's energy saver/doubler.

Administration officials have been working hard behind the scenes, hoping the Senate can be persuaded to mitigate some of the worst effects. Unless the funding prospect improves, there will "really be some sort of chaos and disaster", warns Dr Edward Frieman, director of DoE's Office of Energy Research, predicting in particular that up to 1,500 scientific and technical jobs could be lost at the national laboratories.

So far, nuclear physicists seem to be accepting what is in store for them phlegmatically. Almost two-thirds of the 76 university-based accelerators operating in 1970 have since had to close, many for lack of operating funds.

There is also widespread concern that these developments are putting off potential research students, while the supply of postgraduate research workers is also beginning to dry up. One estimate is that there are currently several hundred vacant teaching and research assistantships.

DoE officials still hope that a reasonable programme can be salvaged. Internal figures being used in planning next year's budget include an increase in nuclear physics funding from \$115 million to \$130 million between 1982 and 1986 (in 1980 dollars) — in contrast to level-pegging for high-energy physics.

But nobody pretends that this year's tide can easily be turned. Mr Robert N Giaimo, chairman of the House Budget Committee, told a meeting of the American Association for the Advancement of Science last week that in the near future "there has to be more discrimination between basic research which is likely to be useful and basic research which is not likely to be" — and that faced with the prospect of a non-expanding pie, the competition for funds will get increasingly fierce.

David Dickson

Heidelberg lab

Danes drag feet

A GROUP of Danish scientists who advise the government on the scale and division of Denmark's basic research budget has recommended that Denmark should no longer contribute to the costs of the European Molecular Biology Laboratory, based in Heidelberg. If the Danish government were to accept the advice, it would be a severe blow to the morale — if not the pocket — of the £10 million a year laboratory, which was launched by an

agreement of ten nations in July 1974 and opened in May 1978.

Denmark was obliged to make a decision on EMBL this year. So far, its Natural Science Research Council and its Agricultural and Veterinary Research Councils have been supporting EMBL (to the tune of some £120,000 a year) out of their own budgets; but it had been agreed in July 1974 that this arrangement should last for only seven years. The source in Denmark of the EMBL contribution for 1981 thus came into question.

Normally in Denmark, subscriptions to international bodies are paid out of government rather than research council funds — that is to say, the sums are allocated from the total research budget before it is divided among the various research councils. So when a committee of scientists from the Natural and Agricultural Research Councils considered who should pay for EMBL in 1981, it attempted to transfer the burden to what it considered to be the rightful place — at government level, but effectively shared among all the other research councils.

None of this need have caused a stir in Heidelberg. In making its administrative recommendation, however, the joint committee chose also to criticise Danish participation in EMBL. For example, the committee pointed out that there is no Dane at the laboratory, and that its programme of research seemed to be rather disjoint from Denmark's.

When this rather qualified recommendation went to the government's science advisory body, the body decided against further participation in EMBL, in the light of tight financial constraints imposed upon it by government.

Denmark thus finds itself in a difficult position. The agreement establishing EMBL was reached as a treaty by West Germany, Austria, Denmark, France, Israel, Italy, the Netherlands, the UK, Sweden and Switzerland, and abrogating it would be a diplomatic matter, as it would be for a nation leaving the high-energy physics laboratory, CERN (on which the EMBL constitution was modelled). However, Danish scientists believe the money will be found somehow, probably by a small group of research councils footing the bill independently, somewhat as before. The new arrangements will have to be complete by mid-October, in time for the Finance Committee of the Danish parliament to consider them in November.

Robert Walgate

Helsinki agreement

West looks East

Brussels

The whole system of West-East technology transfer needs re-examination, according to Charles Levinson, secretary-general of the International Federation of Workers in the Chemical Industry. This

was one of the few clear ideas to emerge this week from the review by the European Parliament's Political Affairs Committee's of the Helsinki agreements.

Levinson described West-East technology transfer as an inevitably expanding process without any political control of interdependence between Western enterprises and Eastern state trading corporations. The Western entrepreneurs, he said, were providing credits for transfer of capital technology which could then only result in the flooding back of joint production onto Western markets. However, Levinson's grasp of the situation was not echoed by many of his colleagues.

At an international hearing of this kind, it might be supposed that apparent naivety was intended simply to stress a point on the record. However, Dr Guido Carli, former director of the Bank of Italy and now president of UNICE, seemed unaware of several contradictions. Speaking on what the Helsinki agreements call Basket II (economic, scientific, technological and environmental exchange) he accepted unquestioned the CIA estimates that the Soviet Union would become a net importer of energy in 1985 (a forecast which the Soviets emphatically deny).

On the other hand, he failed to rise to a question from the floor on the possible economic threat of increasing West German dependence on gas imports from the Soviet Union. Carli proposed a joint research project into energy saving between the major energy importers, including the Soviet Union, as a future counter to OPEC, yet showed no firm grasp of the statistics and parameters involved.

Professor Stefano Silvestri, of the Institute of International Affairs in Rome, suggested that the EEC's lack of a common "or even serious" energy policy and its consequent dependence on Middle Eastern oil was essentially another aspect of the political and military instability of the Mediterranean area. In general the Mediterranean featured largely in discussions, perhaps because of the presence of Willy Brandt, initiator of the North-South Report on sharing of world resources.

Linkage of the three Helsinki baskets — defence, trade and human rights — is a fundamental of Western policy. Max van der Stoep, former minister of foreign affairs of the Netherlands, stressed that dialogue does not have to be "music in the ears of the Eastern states".

The problem of Sakharov, other Soviet dissidents and Charter 77 signatories must, he said, be raised at Madrid. Linkage between Basket II, trade and technology, and Basket I, defence, normally comes to the fore in connection with the transfer of militarily sensitive and strategic technology.

However, Colonel Jonathan Alford of the International Institute for Strategic Studies in London illustrated another

aspect of that problem. The Western observers at Warsaw Pact manoeuvres, present under the terms of the Helsinki accords, had, he said, been virtually incapacitated by maps drawn to an inappropriate scale and binoculars which somehow failed to work.

Vera Rich

Biotechnology

Products work

Another hurdle has been cleared in the pursuit of profitable products from genetically manipulated bacteria. Evidence presented to the Food and Drug Administration in Washington at the beginning of June makes it clear that at least one bacterially produced human hormone has the biological activity of the natural product.

It was no coincidence that the evidence emerged when and where it did, for the aim was for the FDA to decide whether there is a need for special requirements before the licensing of bacterially derived hormones and other peptides or proteins, particularly for clinical use. Clearly the more evidence there is for the identity, not only in structure but also in activity, of bacterial and natural products, the less necessary will be additional requirements for bacterial products.

Not surprisingly, it was the San Francisco company Genetech Inc., one of the first and most voluble of the biotechnology companies, which produced the crucial evidence. Genetech's evidence centred on human growth hormone (hGH), which has been produced by Genetech bacteria for at least the past year.

When Genetech published its first account of expression of the gene for hGH in *Escherichia coli* (*Nature*, 281, 544; 1979) there was no evidence that the hormone had the biological activity of natural hGH. Now Genetech has at least a solid foundation for that evidence.

The mainstay of their new claim is a comparative study of the stimulation of the growth of rats without pituitary glands by hGH from bacteria and from human pituitaries. The overall weight gains and the tibia growth with either hormone or with a mixture of them were virtually indistinguishable. In a further test, the bacterial hGH appeared, if anything, more potent than the pituitary hGH — an observation which Genetech attempted to relate to analytical evidence that the bacterial hGH is the purer of the two.

Genetech has also explored the possibility that its hGH-producing bacteria would stimulate the growth of rats without pituitary glands when simply present in the gut. A positive result might have rekindled the debate about the hazards of recombinant DNA. In the event, the result was negative. The rats put on no weight even though some of the bacteria survived for several days within the intestinal tract.

Genetech also revealed to the FDA that, in collaboration with Hoffman-La Roche Inc., it had successfully carried out a preliminary test of the biological activity of bacterially produced interferon. Because of the limited quantities of bacterial interferon available, the test was confined to showing that three animals survived infection by a potentially fatal virus when given the drug.

The importance of this test, if confirmed, is that it would establish the activity of bacterially produced interferon even though the carbohydrate groups of the natural product are missing.

Workers from the European company Biogen, in their own paper on the construction of interferon-producing bacteria earlier this year (*Nature*, 284, 316; 1980) expressed concern about the possible inactivity of the bacterial interferon. "If a lack of appropriate glycosylation diminishes the activity of the molecule, we shall have a problem on our hands". Their rivals at Genetech seem now to have provided a measure of reassurance on that score.

Peter Newmark

Soft money jobs

Policy rebels

The UK Science Research Council Astronomy II committee decided last week to ignore the SRC's six-year limit on short-term contracts in its future consideration of grant applications. The 25 members of the committee, which deals with infra-red, optical and ultra-violet astronomy, decided that they would advise senior researchers to "appoint the best person for the job" even if the potential researcher has been supported for more than six years on soft money.

The committee intends to consider applications for projects employing researchers whose time would run out before completion as well as applications involving the employment of a researcher who has already exceeded the SRC's upper limit of two three-year contracts.

The committee is taking its action "as a temporary measure" until a formal policy is evolved. Applications will be dealt with case by case, and the committee will fight for its recommendations, expected to be positive in all but borderline cases, on the Astronomy, Space and Radio Board, the next step up in the SRC hierarchy. The committee also empowered the Royal Astronomical Society to act on its behalf in a "big" meeting with the council to take place some time in November.

The committee's action comes at a time when the lack of university openings and requirements of modern astronomical research for specialized technology are placing a premium on retaining experienced researchers in temporary jobs. The SRC's policy, enacted in 1975, had been loosely applied until last November when

the SRC circulated a memorandum instructing its research directors to apply "rigidly" all rules on postdoctoral employment (*Nature*, 7 February). Since then, senior researchers have had their research programmes disrupted and a number of physicists have been forced out of work or have left the country. "It is a major cock-up all along the line. We are sure it is going to be reversed eventually" said committee member Professor Mike Disney of University College, Cardiff.

The case of Paul Gough, a physicist at the University of Sussex, is cited as one example of how the restriction is preventing good experiments from being carried out. Gough, whose final contract will expire in 1982, responded to an emergency call for experiments issued by the SRC on 16 April to fill vacant space created by US withdrawal from the Swedish Viking satellite. Gough proposed a novel experimental technique to study magnetospheric particle bunching by on-board data analysis of information collected from other magnetosphere experiments. Data on particle correlations, normally lost because of telemetric limitations, can be sent in analysed form through existing telemetering systems. The experiment is intended to provide information on links in the magnetosphere. Gough has been told by the SRC that the project was acceptable and would be proposed to the Swedish experimenters but that he would not be permitted to work on it because the work would carry him past his 1982 limit. The SRC has no alternative candidate to do the work. Other proposals that have received similar treatment recently include a cometary probe, a study of moon rocks and a proposal to study incoherent radio scattering from the aurora.

The SRC admits that the policy is presenting difficulties. Brian Oakley, Secretary of the SRC, says "it is a choice of evils, but we believe the restriction is best for science as a whole. The young man is important because of his freshness in comparison to the experience of the older man." Oakley also points out that SRC has created a small number of advanced fellowships (60) to assist the estimated 3,000 researchers affected by its decision and has begun to investigate the situation in an attempt to "free the log jam" caused by the lack of university openings.

Underlying the SRC's policy is the widespread assumption that the major contributions are made by younger physicists. The glamorous figures of quantum physics — Pauli, Heisenberg, Dirac — add fuel to the belief that the newcomer just around the corner is somehow going to change the course of an investigation overnight. As Brian Oakley says, "it stands to reason doesn't it? Once mental images get fixed they are extremely difficult to break."

The problems of people on short-term contracts are increasingly recognized to be

a symptom of the strains on the UK's dual support system for the support of research, exacerbated by the lack of tenured jobs in university faculties. The SRC said however last week that it had had an encouraging response to its advertisement to university vice-chancellors of a scheme whose effect would be to help older faculty members to be replaced by younger scientists.

Joe Schwartz

Nuclear Iraq

Plan set back?

The murder of an Egyptian nuclear physicist in his Paris hotel on 14 June has raised questions about the implications of a nuclear pact between France and Iraq, signed in 1975.

The Egyptian, 48-yr-old Dr Yahia el Mashad, was, according to Israeli radio, "one of the rare Arabs commanding authority in matters of nuclear energy". Trained in Alexandria, the United States and Moscow, he was playing a central role in the construction of two research reactors, based on French know-how, a short distance from Baghdad.

The reactors are designed to use, 93%-enriched uranium, a bomb-grade material. The project is under International Atomic Energy Agency safeguards but, said Israeli radio, the death of Mashad will make it "very difficult for Iraq to continue its efforts towards the production of an atomic bomb".

Iraq is the second largest supplier of oil to France, furnishing 18% of her needs; and when the Vice-President of Iraq's Revolutionary Council, Mr Saddam Hussein, requested access to nuclear technology during a visit to Paris in 1975, it was rapidly granted.

The agreement is worth £145 million to France, and involves the construction in the desert of a large thermal "swimming pool" research reactor (similar to Osiris at the French research centre Saclay). Called Tamuz I, it was due to be charged with 13 kg of highly enriched uranium at the end of 1981, and is already under construction. A second much smaller reactor (0.8 MW) is also to be supplied, creating a research centre for some 600 engineers and scientists.

This is the second time that the project has been threatened. Last April, explosions at the naval construction yard of Seynes-sur-Mer damaged metal casings destined for the reactors, and Iraq accused the Israeli Secret Service and the Central intelligence Agency of complicity in the incident. Israel has certainly been concerned about the deal; a few days before Meshad's murder, General Yeoshoua Saguy declared that Iraq would probably have an atomic weapon by the mid-1980s "which will create a totally new situation in the region". Certainly the motive does not seem to have been criminal. Meshad was left with 1400 French francs in his wallet.

Robert Walgate

Harvard finances

Half power-plant

Washington

What started as an attempt to save money by cogenerating steam and electricity for the Harvard Medical School complex is threatening to turn into one of the biggest white elephants in the history of the university.

Construction delays, cost over-runs and disagreements about the dangers to the local community from nitrous oxide emissions have more than quadrupled the original cost of \$50 million. The university has had to dig deeply into its capital reserves; and this in turn could squeeze support for other projects more directly related to teaching and research.

The university first proposed in the early 1970s to build a Medical Area Total Energy Plant (MATEP) to replace its 74-year-old steam and chilled water generator. The plant has been designed not only to provide hot water and air conditioning to a dozen medical and educational institutions in Boston's Longwood medical area, but also to produce electricity for these institutions with six 7000 kilowatt diesel-powered generators.

Once construction costs had been paid for, argued the university, the 30 per cent extra energy that cogeneration provides from a gallon of oil would save more than \$2 million a year. But it reckoned without the rapid increase of oil prices, the effects of inflation on construction costs and community opposition to a new power plant in a heavily-populated area of Boston.

Public opposition has come to focus on the potential dangers of short-term exposure to nitrous oxides. This had been of little concern when the plant was planned, but was raised in a 1976 report from the World Health Organization as a

possible health hazard from diesel-powered automobile exhausts.

The Massachusetts Department of Environmental Quality and Engineering ruled in 1978 that the steam/chilled water part of the plant could proceed. But in the absence of federal standards covering short-term nitrous oxide exposure (still awaited from the Environmental Protection Agency), refused to grant a permit for the diesel engines.

Critics of the plan claim that short-term exposure to as little as 200 micrograms per cubic metre can be harmful. While not accepting this figure, the DEQE ruled that emissions should not exceed 320 micrograms (based on a hearing officer's conclusion that harmful effects have been observed in laboratory animals at exposures of 940 micrograms per cubic metre and his use of a safety factor of three).

Though disputing the scientific evidence, Harvard has accepted this figure and has drawn up a plan showing ways in which it can comply for more than 99 per cent of the time.

Following further hearings, the DEQE has now accepted most of the university's arguments about the plant's safety and rejected protesters' claims that carcinogenic hazards require further investigation.

However, the state's Deputy Commissioner, Mr David Fierra, two weeks ago again refused an operating permit on what Mr Edward Lashman, Director of Special Projects at Harvard, calls a "single, highly technical issue". This is whether, under certain operating conditions, the plant will endanger public health by contributing further to already high concentrations of nitrous oxides arising from traffic exhaust at a number of congested "hot spots" in the area.

The university is now seeking a further hearing, claiming that the 320 microgram limit will be exceeded at these locations for only 20 out of 8,760 hours in the year, and

No power for the patients?

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

raising the question of "whether the frequency of adverse diesel impact will be significant enough to justify disproving the diesels".

University officials are confident that they will win their case. But even if the plant is given permission to operate — and local community groups are keeping up strong opposition — the university already faces a large bill resulting from the delays so far.

The latest cost is estimated at about \$215 million. Attempts to borrow this money have failed, largely because potential investors have been scared off by the regulatory uncertainties and the unwillingness of potential customers to sign long-term contracts into which the extra costs have been absorbed.

Furthermore, the university is unlikely to be allowed to raise its charges for electricity to hospital facilities to a rate higher than that charged by the local utility company, Boston Edison, therefore having to subsidize the power it provides for some time to come.

With a total endowment of \$1,400 million, there is no danger of bankruptcy. However, university officials emphasize that they have no intention of using existing endowment funds — or the extra \$250 million in capital reserves being sought in a fund-raising campaign — to cover the costs of MATEP.

Unless the money can be borrowed from elsewhere, the result will be intense pressure on the university's general operating account, which has already had to cover \$170 million in construction costs.

David Dickson

European space

Meeting Halley

In spite of worries about two of its most highly-prized projects, the European Space Agency is giving some attention to the more cheerful prospect of a fly-by mission to Halley's comet in the mid-1980s. This has the support of solar system physicists throughout Europe, although approval would rob high-energy astrophysicists (increasingly sensing neglect by ESA) of a mission to meet their needs.

The agency's scientific advisory committee, which gives advice on the scientific merits of proposals and which includes high-energy astrophysicists among others, came out strongly in favour of the cometary mission last week. This is, after all, the only opportunity for 76 years to investigate the physics of the solar system's most notable comet. Before the final go-ahead can be given, the mission will have to be approved by the committee of official delegates of ESA's eleven member states at their meeting early in July.

The chief objective of the mission would be to investigate the materials in the tail region. To this end, the spacecraft would be equipped with mass spectrometers for the measurement of neutral and charged atoms and simple molecules.

Most probably the project will be approved, although some delegates from countries with strong astrophysics communities (for example the Netherlands) and France may take some persuading. The Soviet Union and Japan are also considering Halley missions but the ESA is now the only hope of a Western mission to the comet; the US National Aeronautics and Space Administration abandoned its plans for such a mission some months ago, after budget cuts by Congress. In the circumstances, US scientists and NASA are understandably keen on cooperating with the Europeans. In the past few weeks, this prospect has been dimmed by the uncertainty about the joint ESA-NASA solar polar mission, one of the projects threatened by Congress.

Last week, however, Congress agreed that the international solar polar orbiter should remain as part of the NASA budget after the personal intervention of Dr Frank Press, the President's Science Advisor, and under pressure from the State Department.

ESA's current plan is less ambitious than that widely discussed last year to send one ESA and one NASA spacecraft to both the Halley and Tempel 2 comets. That would have cost about 500 million accounting units (about £315 million). The new plan will cost substantially less, chiefly through the use of the Geos 3 technology.

The European spacecraft would be launched by the Ariane rocket, probably with another satellite heading for a geosynchronous transfer orbit. Confidence in Ariane, however, has ebbed slightly since the failure of the second test flight last month and the option of using a US TD

launcher is being considered. (Geos 3 is incompatible with the space shuttle.) The future of the TD launcher, however, is in many ways even more uncertain than that of Ariane. If NASA sorts out its problems with the space shuttle soon, it might have ceased to operate TD launchers by 1986, the latest date for the Halley launch.

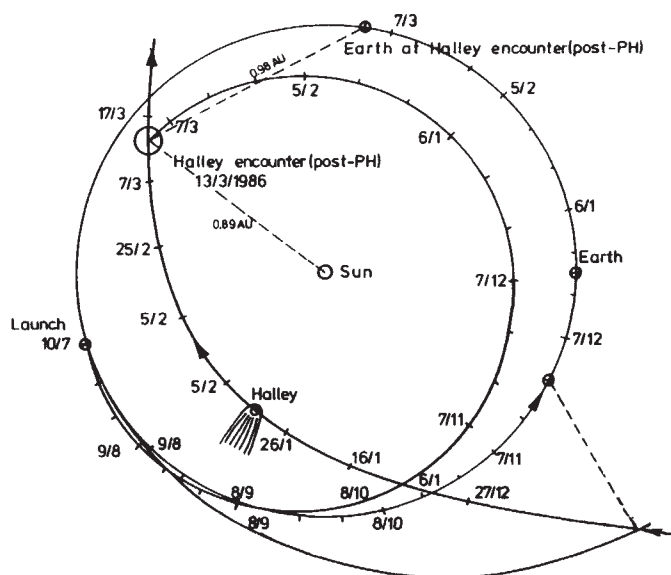
With so many uncertainties, ESA is unlikely to decide on collaboration with NASA until the end of this year. Part of the deal would be that NASA should supply a deep space network to track the spacecraft and to relay data.

Sending a spacecraft to a comet is a risky business. Launching it on Ariane with a satellite to be put into a geosynchronous transfer orbit would reduce the launch window to only two weeks. The timing would be even finer when the spacecraft reached the comet: the most valuable data would have to be collected in a matter of minutes. The encounter would be brief — relative velocities between the comet nucleus and spacecraft would be about 60 km/sec — while positioning and tracking would have to be accurate enough to avoid collision with the cometary nucleus.

The planned flight path approaches within 1000 km of the nucleus on the tail side, so there would be also be a fairly high risk of damage from dust particles or micro-meteorites. Nevertheless, both space scientists and technologists believe there is a reasonable chance of success. A decision to go ahead would affect the pattern of other ESA missions. Within the budget limits, ESA would have to wait until the beginning of 1982 before starting on its next project — possibly a mission to observe transient phenomena in X-ray sources or POLO, the Polar Orbiting Lunar Observatory. Hipparcos, the astrometry mission approved earlier this year, would also have to be postponed for six months.

Judy Redfearn

Orbit of comet Halley with planned track of spacecraft



CORRESPONDENCE

Plan denied

SIR, — In your issue of 5 June you describe under "One way ahead for British biotechnology" a plan by the directors of "three renowned molecular biology laboratories" including the Medical Research Council's Laboratory at Cambridge. To set the record straight there is and has been no plan of the sort you describe, although the three scientists you name have, in common with many other experts, been consulted by the National Enterprise Board as part of that body's concern with biotechnology.

If the NEB were to set up a company to help exploit British skills in biotechnology — and the Medical Research Council has already warmly endorsed the recommendation in the Spinks' Report for such an initiative — any role the Council might play in it would involve far more than the work in the Cambridge Laboratory (distinguished though that is) and will, of course, be negotiated by the Council in consultation with all its relevant Unit directors.

Yours faithfully

J. L. GOWANS
Secretary, Medical Research Council
London, UK

Controversy buried

SIR, — The adversarial approach to resolving matters of public importance has its uses, but where those matters turn upon facts, which must be acquired and interpreted, it can be counter-productive. The attempt to clarify issues by oversimplifying them polarizes the arguments and the truth appears as the opinion of the dominant faction. It becomes difficult and sometimes impossible to express an alternative view. In recent years we have seen the controversies over cyclamate sweeteners and pesticides polarized in this way and today the media presentation of the "debate" over nuclear power amounts to little more than a statement of the anti-nuclear case.

While journalists are much to blame, scientists themselves may contribute to the confusion either by retreating into a splendid isolation while the information they have obtained is used responsibly or irresponsibly by propagandists, or by joining the fray, perhaps unwittingly, and seeking data to support the cause of their choice. In itself this is harmless enough, and science often proceeds by accumulating evidence to support an attractive hypothesis, while contradictory evidence, which eventually destroys the hypothesis, is obtained more slowly. Where a polarized argument creates a false sense of urgency, however, so that important decisions are made on the basis of partial information, the public interest is not served. The history of the US Environmental Protection Agency contains many examples of such hasty decisions and recommendations.

The latest victim of adversarial skirmishing is the debate over the effects of chlorofluorocarbon (CFC) compounds on the ozone layer. As a writer and a scientist we have tried to suggest that the ban on most aerosol propellant uses for CFCs cannot be justified by the evidence that is available. We have a case, or so we believe. While we do not quarrel with the general proposition advanced by Rowland and Molina, that CFCs can destroy ozone, we note that two-dimensional atmospheric models suggest that much of the resultant depletion will occur over the arctic during winter, when lost ozone is not replaced. We are not convinced that CFCs could

endure indefinitely in the troposphere, yet a CFC sink here would reduce the ozone depletion substantially. Nor do we regard it as certain that a small increase in UV radiation at the surface would be malign. The basis of the view that UV radiation is highly dangerous — and hence of our concern over the ozone layer — is that advanced organisms could not evolve, and dry land could not be colonized, until sufficient oxygen had been released by photosynthesizing organisms in the seas to allow an ozone layer to form. There is no evidence to support this view and it is challenged strongly by many biologists. Probably there is a causal relationship between exposure to UV and nonmelanoma skin cancer in humans, but the link between UV and melanoma is too weak to be convincing. Finally, we would point out that if CFCs are so dangerous as to warrant banning, pressure will soon build to extend the aerosol ban to other uses of CFCs in refrigeration, insulation and foams, for which we may incur an energy penalty as well as an economic one.

Our views are hardly revolutionary, but we have had difficulty in expressing them in public. An article has been commissioned from us, so we have no grounds for personal complaint, but an earlier article that had been commissioned was rejected on the advice of American referees and another offer of an article was politely declined. We know of other writers who have experienced similar difficulties in criticising the CFC ban, and we believe that contrary views are being suppressed while truth is dictated by fashion.

If we are committed to decision by combat it is rather important that those who make the decision be exposed equally to all arguments and that we find some way to give weight to the view that the information available justifies no decision at all. We fear that by crying "Wolf!" repeatedly over supposed environmental threats, one day we may not be heard when the threat is real. Indeed, it is a curious fact that amid all the quite genuine threats to the global environment about which we do possess information, the purely theoretical CFC threat to the ozone layer is the one that has resulted in legislation. We may embrace the adversarial method or we may reject it, but we must not distort it in this way. If we are to embrace it, scientists must be prepared to participate and journalists and their editors must assist them to do so.

Yours faithfully,

MICHAEL ALLABY
Wadebridge, UK
J.E. LOVELOCK
Launceston, UK

Discussion stilled

SIR, — As the author of one of the reports "which have become the centre of fierce controversy in the climate research community" (*Nature*, 8 May), I feel compelled to respond briefly to your short piece on the possibility that increased atmospheric CO₂ concentrations could lead to increased tensions between those nations of the world classified as rich and poor. It comes as no surprise to me that the special committee of the National Academy of Sciences that issued the report with this conclusion agrees with the experts consulted that our dissenting reports are "based on incomplete assessments that unrealistically omit important feedback processes".

Our findings that the greenhouse effects of atmospheric CO₂ are a full order of magnitude less than the previous scientific consensus are so much at odds with the thinking of the past

several decades that it will be some time before they receive a dispassionate and objective evaluation. This is particularly so because they are based essentially on experiment, whereas most earlier work in this area has been of a theoretical nature. It is therefore doubly curious that the experts claim we have omitted important feedback processes for we have dealt with the real atmosphere, measuring the effects of whatever feedback processes of a significant nature are occurring in real time.

I would thus like to encourage those experts that disagree so violently with our findings to submit their judicious appraisals of our work¹ for publication, where they can be answered in a public forum.

Yours faithfully

SHERWOOD B. IDSO
Tempe, Arizona, USA
¹Idso, S.B., *Science*, **280**, 1462; (1980).

Engine exhausts

SIR, — David R.L. Davies (17 April), points out that automobile combustion chamber deposits accumulating as a result of the use of leaded petrol cause an increase in octane requirement. Unfortunately, he implies incorrectly that deposits from unleaded petrol do not have such effects. In fact, combustion chamber deposits from unleaded petrol generally cause greater increases in octane requirements than those from leaded fuel.

Of the several investigations of this subject, the work of the US Coordinating Research Council (CRC), which involved contributions from 15 major US laboratories (auto manufacturers, fuel suppliers, etc.) is the most extensive. They reported that their data "indicate that cars operated on fully leaded fuels have less Octane Requirement Increase (ORI) than those cars operated on unleaded or low lead fuels". A summary of the work by the CRC was presented before the US Society of Automotive Engineers in 1973 by Bigley and Benson (SAE Paper 730013) who reported the average ORI effect was 2.0 to 2.5 research octane numbers *higher* for unleaded than for leaded petrol.

To avoid knocking complaints for a given percentage of vehicles, the octane quality of unleaded fuel must be increased above that of the leaded petrol which it replaces. This increases the cost and crude oil consumption penalties associated with unleaded petrol.

Yours faithfully

WILFRED E. BETTONEY
E.I. du Pont de Nemours & Co., Inc.,
Wilmington, Del., USA

SIR, — In response to my earlier correspondence (17 April) on lead based petrol additives, combustion chamber deposits and fuel efficiency, Wilfred E. Bettoney points to tests conducted by the US Coordinating Research Council in 1972. My understanding of the tests by the US CRC is that they compared leaded petrol containing additives to reduce and modify deposits of lead compounds with unleaded petrol where no effective effort was made to reduce carbonaceous deposits.

In any case, tests carried out on the 1971 US vehicle fleet are not directly relevant to European or Japanese vehicles designed since 1973. To be more specific, recent developments such as electronic ignition control and a shift to lean fuel mixtures have reduced both the need for high octane fuel and the rate of carbon deposition.

Yours faithfully

DAVID R.L. DAVIES
Centre for Resource and Environmental
Studies, Australian National University,
Canberra, Australia

NEWS AND VIEWS

Heat flow and the deep structure of the continents

from Philip England

SOME seismologists now believe that the plates which form the mobile outer skin of the earth differ strongly in their thermal and mechanical properties between continent and ocean, these differences extending to depths of 400 km or more⁹. If this is true, the implications for the chemical evolution and mechanical stability of the continents are profound. Unfortunately, the seismic data are scanty and the interpretations controversial, so constraints from an independent set of data would be valuable. Recently, Vitorello and Pollack⁷ have argued that measurements of surface heat flow on the continents provide just such an independent constraint, and that it too requires that continents have 'roots' more than 300 km thick.

It has been recognised for some fifteen years that conductive heat flow from the surface of the continents is related to the geologic age of the crust. Developing from the observation that heat flow from Precambrian (more than 600 Myr old) provinces is generally lower than it is from younger regions¹, it has now become generally accepted that there is a monotonic decrease in the surface heatflow with increasing age of the crust, on a timescale of 200–500 Myr^{2–5}.

The study of heat flow from the ocean floor, and of ocean bathymetric data which are closely linked to heat flow, has provided crucial insights into the origin and development of the oceanic portions of the plates. The surface heat flow and the elevation of the oceanic crust both decrease with age and approach steady state values when the crust is 70–100 Myr old. These observations can be explained in terms of the cooling of the upper mantle from a condition of partial melting at the mid-ocean ridges to one of near thermal equilibrium in the old oceans, where a cool, and therefore rigid, mechanical boundary layer some 100 km thick overlies a convecting upper mantle. The dominant mode of heat transport in this layer — the lithosphere — is by conduction.

The directness with which interpretations of oceanic heat flow led to improved understanding of the thermal and mechanical nature of the oceanic lithosphere has naturally resulted in similar attempts to analyse continental heat flow.

However, the determination of heat flow on land — and of its relation to the age of the crust — is far more difficult than the corresponding tasks at sea. Continental temperature measurements are confused by such effects as topography, ground-water flows and conductivity contrasts. Whereas the age of the oceanic lithosphere is clearly given by the date of solidification of its basaltic crust (a process occurring over a few million years at most), the tectonic process affecting continental crust take considerably longer (100 Myr is not an unusual duration for an episode of mountain building) and may strike the same piece of crust more than once in its history.

Nevertheless, the overall picture we have of heat flow decay on the continents, although originally derived from less than 150 observations², has changed little now it is based on 1,700 observations⁵. When the heat flow measurements are grouped according to the time elapsed since the last major tectonic event to affect the crust they show a decay of continental surface heat flow from an average of around 80 mWm⁻² in terrains less than 200 Myr old to an average 60 mWm⁻² in terrains 300–400 Myr old with an approximately steady average heat flow of 45–50 mWm⁻² for terrains in the range 600–2,500 Myr old. It must be emphasized that these data exhibit great scatter, much of which results from the uncertainties mentioned above. In addition, a significant proportion arises from a complexity not present in the oceanic case.

In the oceanic crust the proportion of radioactive isotopes present is very small, and they contribute less than 5% to the surface heat flow, but on the continents, the decay of radio-active isotopes is responsible for around 50% of the surface heat flow. These radiogenic elements are unevenly distributed within the crust and thus the surface heat flow in a province of a given age can vary considerably, just because of changes in the intensity of near-surface heat production, without in anyway indicating variation in the deep thermal structure of the region.

Philip England is IBM Research Fellow in the Department of Earth Sciences, Bullard Laboratories, Cambridge University.

The scatter of heat flows measured within provinces of given age spans are greater than the range of the means from provinces of all the different age spans. It is thus natural to ask whether the variation in continental heat flow is the result solely of the variation of conditions within the crust, or whether the slow decline in heat flow with age reflects some deeper seated thermal process within the continental lithosphere.

In the past, the latter class of explanation has been favoured and the thermal development of the continental lithosphere has been explained in terms of cooling from an initial high temperature regime, with erosion playing a comparatively minor part by removing near surface radiogenic heat sources.

The most recent and most thorough explanation of this type has been given by Vitorello and Pollack⁷. They propose a three component model for the continental heat flow decay: **I** a component due to radiogenic isotopes within the crust, this is in part removed by erosion on a timescale of 300–400 Myr; **II** a component due to the decay of a temperature perturbation caused by the 'tectonothermal' event (e.g. mountain building, plate boundary volcanism, etc.) that now defines the age of the crust; **III** a constant background contribution from the upper mantle. To fit the surface heat flow observations, a decay of some 40mWm⁻² over 300–400 Myr is required. Relying on an empirical relation⁸ suggesting that 40% of continental surface heat flow is generated within the crust (i.e. by component I.) Vitorello and Pollack suggest that 40% of the heat flow decay comes from the removal by erosion of an approximately 10km thick layer of crust rich in radiogenic elements.

The remainder of the secular decay must now come from component II of their model — the conductive decay of the temperature perturbation produced by the 'tectonothermal' event. It is here that the major implication of Vitorello and Pollack's model becomes apparent, for they require this decay of around 30mWm⁻² to take place over 400–500 Myr and show that this requires the conducting boundary layer beneath the continents to be over 300 km thick — three

times the thickness of the corresponding layer in the oceans. For this layer to remain stable, and not become involved in mantle convection, and to explain the absence of the large thermal subsidence (analogous to, but larger than the 4 km difference in elevation between ocean ridges and the abyssal plains) which the cooling of such a thick layer would involve, Vitorello and Pollack suggest that the 'tectonothermal' events which perturb the continental mass result in partial melting of the upper mantle to give basaltic volcanism, leaving behind a cold viscous residue which is reduced in density owing to the extraction from it of the denser fractions which go to make up the basalt.

This model has considerable geochemical and geophysical implications. First, it implies that every 'tectonothermal' event which affects the crust involves considerable partial melting of the mantle, with the transport of mantle-derived melt into the crust and the depletion of some 300 km of upper mantle in iron and a number of other elements — notably the heat producing ones. This bears closely on the argument raging among geochemists as to whether the differentiation of the earth is a continuing process — with new crust constantly being created — or whether the bulk of continental crust was created in intense activity 2,000 to 3,000 Myr ago while the earth was much hotter.

Second, Vitorello and Pollack's requirement of deep, stable continent roots lends support to the claims by a small but vociferous band of seismologists that differences in seismic velocity between continents and oceans extend at least as deep as 400 km (for a recent review see ref. 9). Among other things, this model would also imply that it should be harder to move plates that have large continents on them, because the drag on their 'keels' is much greater than that on the 100 km thick oceanic plates.

This model, then, has intriguing possibilities, and the success of similar interpretations of the oceanic data, makes it tempting to accept Vitorello and Pollack's conclusions. However, the conclusions are model-dependent and in view of their weighty geophysical and geochemical implications it is disappointing that the authors do not suggest a mechanism to account for the far greater depth extent

which they propose for partial melting beneath the continents than beneath the mid-ocean ridges; nor do they give any indication of whether the quantity of partial melting they invoke is consistent with the volumes of basic volcanics associated with present or past zones of activity in the continental crust.

In addition, many geologists familiar with young mountain belts will be surprised at the small length scale and long timescale that Vitorello and Pollack assign to the erosion in their model, 10 km of erosion over 400 Myr is quite small when compared, for example, with the 20 km or so of erosion from the axial zone of the Alps in the last 30 Myr, or the widespread inference, from mineral assemblages, of burial depths of 20 to 30 km experienced by crustal rocks during mountain building episodes throughout geologic time. Richardson¹⁰ has shown that the entire variation of continental surface heat flow can be explained by the removal by erosion of the radiogenic elements contained in 20 km of continental crust. The geological

record suggests that thickening of the crust by 20 km and subsequent erosion by this amount is a common occurrence in zones of continental collision like the present day Alpine-Himalayan chain.

So, there are at least two explanations of the continental heat flow relations, one in terms of a deep-seated thermal 'root' to the continents and the other in terms of purely crustal processes.

While Vitorello and Pollack have produced some persuasive reasons for interpreting continental heat flow in terms of the cooling of a boundary layer 300 km thick, they have not shown that this model is required by the data; other models with lithosphere no thicker than the oceanic one are also consistent with the data^{10,11}, and are in better agreement with our understanding of the evolution of mountain belts.

In conclusion, the controversial seismic observations which seem to suggest that continents have deep roots must await support from some other source than surface heat flow data. □

Cereal yields and drought resistance

from S.A. Quarrie

CEREAL yields in the UK have been increasing by 50-70 kg/ha each year for the past 30 years. They are now about 80% higher than they were in 1950. Improvements in agronomic practices, particularly the increased use of nitrogen fertiliser and crop protection chemicals, account for part of this increase. How successful, then, have the breeders been in improving the yields of cereal varieties and what are the prospects for the future?

Answers to these questions for winter wheat have now been provided by Austin *et al.* (*J. agric. Sci.* **94**, 675; 1980). They compared the yields of old varieties dating back to 1908 with those of modern ones in an experiment in which lodging (bending at or near the soil surface) and severe infections of disease were prevented. On two sites of differing fertility the best of the new varieties consistently outyielded the old varieties by about 40%. At each site the total dry matter production of the varieties was similar. The new varieties, which were much shorter, had lower stem weights than the older ones. So the breeders, in seeking to achieve resistance to lodging by selecting for dwarf habit, have gained a bonus. Because the total dry matter has not changed, reductions in stem weight have been accompanied by exactly balancing increases in grain weight. The modern varieties flower earlier and grain filling occurs over a longer period.

The authors suggest that there is a limit to which stem weights can be reduced with a concomitant increase in grain yield and this would give at most a further 25% yield

increase. Thus, to continue increasing grain yields into the 21st century will require varieties capable of giving a greater biomass yield. Unfortunately, even if the genetic resources are available to do this, an increase in biomass yield is likely to increase water use. But shortage of water is already a factor limiting yields of wheat and barley in the UK. Using data from various irrigation experiments in recent years, Austin (*ADAS Quart. Rev.* **29**, 76; 1978) has estimated that drought causes an average yield reduction of 17% in the UK. The beneficial effect of irrigation was most marked on light soils, but, since irrigation would not be economic for cereals in Britain, it seems clear that in the future greater emphasis needs to be placed on more efficient use of available rainfall and stored water. Although it may be possible to reduce yield losses due to drought by cultural treatments, recent research has suggested ways by which the drought resistance of new varieties may be improved by breeding.

For many years physiologists and breeders have been looking for characters which reliably predict drought resistance, but with relatively little success. The problem is twofold. First, drought itself is often variable both in its severity and timing, and this determines the way and extent to which yield may be reduced. Second, few scientists studying the problem have had adequate genetic material for testing the effects of variation in a single character in a uniform genetic background. Physiologists are now

1. Kraskovski, S.A. *Izv. Akad. Nauk. Arm. SSR Geol. Geogr. Nauki. Eng. Tran.* **247** (1961).
2. Lee, W.H.K. & Uyeda, S. *Geophys. Monogr. Series* **8**, 87 (ed. Lee W.H.K. AGU, Washington 1965).
3. Polyak, B.G. & Smirnov Ya. B. *Geotectonics Eng. Trans.* **4**, 205 (1968).
4. Sclater, J.G. & Franchet, J. *Geophys. J. Roy. Astr. Soc.* **20**, 509 (1970).
5. Chapman, D.S. & Furlong, K. *EOS Trans AGU* **58**, 1240 (1977).
6. Parsons, B. & McKenzie, D.P. *J. Geophys. Res.* **83**, 4485 (1978).
7. Vitorello, I. & Pollack, H.N. *J. Geophys. Res.* **85**, 983 (1980).
8. Pollack, H.N. & Chapman, D.S. *Tectonophysics* **38**, 279 (1977).
9. Jordan, T.H. *Nature* **274**, 544 (1978).
10. Richardson, S.W. in *Geodynamics Today — a review of the Earth's Dynamic Processes*. 123 (Royal Society, London, 1975).
11. England, P.C. & Richardson, S.W. *Geophys. J. Roy. Astr. Soc.* **62** in the press.

working with breeders to create the genetic diversity necessary for this work.

There are some recent examples of this. Jones (*J. exp. Bot.* **28**, 162; 1977) looked at the effect on transpiration of stomatal frequency in related lines of barley bred by Rasmusson. Contrary to expectation a reduction in stomatal frequency did not cause a reduction in transpiration. Instead, the lines with a low stomatal frequency had larger stomata and larger leaves and these differences totally compensated for the reduced stomatal frequency; to decrease transpiration selection for small stomata or small leaves would also have to be carried out.

The development of the root system is obviously of vital importance to the water economy of the plant. In two recent papers (O'Brien, *Aust. J. Agric. Res.* **30**, 587; 1979; Robertson *et al. Crop Sci.* **19**, 843; 1980) the authors have looked at genetic variability in the length and number of wheat roots. Their ultimate objectives were to improve our understanding of the requirements for optimising water uptake in different environments. They found that potentially useful genetic variability exists in the length and number of seminal and lateral wheat roots, and O'Brien is already preparing experimental lines by hybridisation and selection. From studies with these lines it will be possible to elucidate the interrelationships between root growth, moisture uptake and crop yield. Optimum root development patterns can then be defined for various kinds of drought.

The biochemistry of stress is also being studied. The concentration of the amino acid proline often increases several-fold in response to water stress. Work in the early 70s suggested that proline may have a role to play in determining drought resistance in barley. However, Hanson *et al. (Crop Sci.* **19**, 489; 1979) have recently studied proline accumulation in related barley lines selected on the basis of their high or low drought-induced proline accumulation. They found no evidence that accumulation of free proline was of survival value during severe drought and concluded that proline accumulation was essentially a symptom of the development of severe plant water deficit.

The plant hormone abscisic acid (ABA), the concentration of which also increases during drought, may be more directly related to drought responses. Applied ABA has effects on the development and physiology of plants similar to those of water stress. An additional effect of ABA in droughted plants has been suggested by Morgan (*Nature*, this issue p655). Water stress at meiosis reduces seed set and Morgan has shown that applied ABA can also do this. When plants were droughted, ABA concentration in the flag leaves increased and ABA was also transported to

the ears, although these did not develop stress. Several cereal species have now been examined for differences in drought-induced ABA accumulation. Larqué-Saavedra and Wain (*Ann. appl. Biol.* **83**, 291; 1976) looked at related lines of maize known to differ in drought resistance and found that the more resistant lines had a greater capacity to accumulate ABA when droughted.

Flares without acceleration, or acceleration without flares?

from John C. Brown

A basic problem associated with solar flares, after the difficulty of producing sufficiently rapid primary energy dissipation, is that of particle acceleration, a process also observed to occur under other cosmic conditions. Any acceleration problem involves explanation of generating sufficiently high individual particle energies and of acceleration of sufficiently many particles (total particle energy). In principle the former is readily achieved for flares by the electric fields of up to 10^{10} V, which could be induced by the rapid reduction in flare magnetic field, sufficient to account for the most energetic (GeV) ions observed. On the other hand, the bulk of the total energy of flare particles resides in the non-relativistic (≈ 1 –100 keV) electrons detected directly in space and by the hard X-ray (bremsstrahlung) and radio (gyrosynchrotron microwave and Type III plasma oscillation metre wave) bursts they emit in the flare plasma.

Till now most interplanetary electron event observations have been confined to energies $E \geq 10$ keV. At such energies it has been found (Lin *Space Sci. Rev.* **16**, 189; 1974) that while electron events are closely associated with flares, the converse is not true i.e. many flares produce no (detectable) electron event. This is consistent with the notion that particle acceleration is a process which is secondary to a basically thermal dissipation of magnetic energy in flares and is only initiated by a sufficiently large or transient primary release. On the other hand in those flares where acceleration does get under way the ≥ 10 keV electrons often acquire a fraction of the total flare energy sufficiently large to demand a substantial acceleration efficiency, and perhaps to play an important role in transporting energy through the flaring solar atmosphere.

Potter, Lin and Anderson (*Astrophys. J. Let.* **236**, L97; 1980) have now extended the survey of interplanetary electron events down to 2 keV energies using data collected by the International Sun-Earth Explorer (ISEE) 3. They have found that the acceleration of 2–10 keV electrons is a very

Many physiological and morphological characters are significant for water economy, though for a given character the useful variation within a cereal species may be limited. Once the characters which can be exploited to improve drought resistance in cereals have been identified it might be possible for plant breeders to maintain the rates of yield improvement they have achieved in recent decades. □

frequent event, apparently occurring without a detectable electron event at higher energies and in the absence of any reported flare activity as measured by chromospheric ($H\alpha$) events and by radio and X-ray bursts. These low energy electron events, however, seem closely associated (as are more energetic electron events) with low frequency (< 1 MHz) type III radio bursts (also observed by ISEE-3) believed to be due to plasma wave excitation in the low density outer solar atmosphere by the electron stream passage. Potter *et al.* regard these observations as direct evidence for non-flare associated electron acceleration in the corona. While the possibility is not yet excluded that $H\alpha$ and X-ray bursts did occur in these electron events, but below the threshold for detection (or reporting), the data certainly indicate the frequent operation of a dissipation process whose primary product (energetically) is accelerated electrons rather than hot plasma. The possibility that this can also sometimes occur at higher electron energies was suggested earlier on the basis of a survey of indirect electron data by Kane *et al. (Solar Physics* **38**, 438; 1974).

The best venues for production of non-thermal effects are theoretically those where the thermalisation time is as long as possible compared to the duration of energy release — in this case high in the corona where the plasma density is low and collision times long so that electrons escape before they can thermalise. Potter *et al.* argue that this is indeed the acceleration site from which the 2–10 keV electron events emanate, and specifically suggest an altitude of at least 0.5 solar radii ($R_{\odot}$) above the photosphere. Their conclusion is based essentially on the observation that the electron events have spectra extending smoothly down to about 2 keV with no turn-over (though the power-law index does decrease at the lowest energies). It is then argued that if the electrons started out with a power-law spectrum E^{-4} at an

John C. Brown is in the Department of Astronomy, University of Glasgow.

altitude $\leq 0.5 R_{\odot}$ (density $n \leq 10^7 - 10^8 \text{ cm}^{-3}$) collisions with the intervening plasma would turn over the low energy end of the event spectrum seen near the earth. This argument, though attractive, is not watertight. In particular if the acceleration spectra were steeper at low energies then collisions would flatten them toward the observed form. Second, any tendency for collisions to turn over an initially power-law spectrum would be offset by the spectral redistribution due to plasma waves generated by the bump in the electron velocity distribution itself. Nevertheless it seems likely that the argument used by Potter *et al* does indicate the density of the acceleration region correct to order of magnitude and it is of interest to pursue further its physical implications.

First, there arises the question of stability of the electron stream in a plasma of such low density. In particular, the electron flux over an assumed area of $(10^{-2} R_{\odot})^2 \approx 5 \times 10^{17} \text{ cm}^2$ would be about $5 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$, which, in an ambient plasma density of $\geq 10^7 \text{ cm}^{-3}$ will drive a return current with drift velocity $v_D \leq 5 \times 10^6 \text{ cm s}^{-1}$. This is below the ion-sound speed for any reasonable coronal temperature, so that the return current should be ion-sound stable. For these same parameters, Ohmic losses to the beam in driving the return current should also be small compared to the direct collisional losses, as tacitly assumed in the Potter *et al* analysis. Second, the magnetic field strength at these high altitudes will be low but, as Potter *et al* point out, still adequate to supply the total electron energy by annihilation of a 1 gauss field in a volume of $(10^{-2} R_{\odot})^3$. Third, at a density of 10^7 cm^{-3} and temperature around 10^6 K , the electron collisional relaxation time is several seconds for thermal electrons and several tens of seconds for 2 keV electrons. As indicated above such time scale conditions are favourable for release of energy primarily into nonthermal forms.

It appears then that pure acceleration of (low energy) electrons in the corona without chromospheric flare effects may be a common occurrence and as potentially important an acceleration diagnostic as flares themselves. For higher energy electrons to be accelerated, magnetic fields and gradients must presumably be stronger, so that lower altitudes and correspondingly higher densities will be involved. The shorter thermalization times would then lead to substantial heating of the coronal plasma (soft X-ray flare) and the combined effects of downstreaming electrons and of thermal conduction would also produce chromospheric flaring. Further elucidation of the relative importance of heating effects associated with acceleration of electrons of different energies and fluxes, should emerge from the current Solar Maximum Mission with its wide spectral coverage and coordination with other space, and also ground based, experiments. □

Early steps in excision repair

from A.R. Lehmann

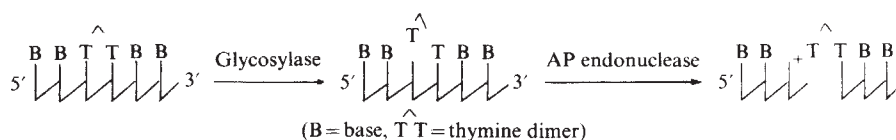
WHEN excision-repair of DNA damage was discovered by Setlow and Carrier in 1964, they suggested that it could be carried out by four enzymatic steps: an endonucleolytic nick was made in the vicinity of the damage, the damaged section was removed by an exonuclease, the resulting gap filled in by a DNA polymerase, and the new stretch of DNA joined to pre-existing DNA by ligase. Though this model remains correct in outline, the details of excision repair have turned out to be much more complex in detail, the whole process being under the control of numerous gene products both in *Escherichia coli* and in eukaryotic systems. A variation of the classical scheme of 'nucleotide excision-repair' was discovered some years ago, when it was found that a number of altered bases in DNA such as uracil, hypoxanthine and 3-methyladenine were excised in a different manner, termed 'base excision-repair'. The first step in the removal of these bases was not the scission of the phosphodiester backbone, but the rupture of the glycosylic bond between the altered base and the deoxyribose sugar. The resulting AP (apurinic or apyrimidinic) site in the DNA could then be removed by sequential action of an AP endonuclease (found ubiquitously) and exonuclease.

The major type of damage produced in DNA by UV light is the pyrimidine dimer, resulting from the formation of a cyclobutane ring joining two adjacent pyrimidine bases. This lesion causes a large distortion of the DNA helix, and it has been studied more extensively than any other type of DNA damage. Until very recently the pyrimidine dimer was thought to be removed by nucleotide excision repair rather than base excision repair because pyrimidine dimer-specific endonucleases, purified from either *Micrococcus luteus* or T4-infected *E. coli* seemed to make endonucleolytic nicks on the 5' side of pyrimidine dimers. This mechanism now seems to be incorrect, as shown by work published in this issue of *Nature* (Haseltine *et al.* p634) and by several papers presented at the recent NATO-EMBO Advanced Study Institute on Chromosome Damage and Repair (Godøysund, Norway, 27 May-5 June, 1980). Haseltine and coworkers were investigating the specificity of the endonuclease from *M. luteus* by using a sequenced piece of DNA, and comparing the cleavage sites after UV-irradiation and treatment with endonuclease, with those produced by

specific chemical digestion. The results of these experiments showed that if the starting DNA was labelled at the 5'-end, the enzyme-digested fragments were one nucleotide longer than would be anticipated if the enzyme cleaved the DNA on the 5' side of the pyrimidine dimers. In other words the cleavage site appeared to be on the phosphodiester backbone between the two dimerized pyrimidines. Such a cleavage would in itself be of no obvious advantage in removing the pyrimidine dimer, and the authors went on to demonstrate that this was in fact the second step of the reaction, the first step being the rupture of the glycosylic bond between the 5'-pyrimidine of the dimer and the deoxyribose sugar, leaving an apyrimidinic site. This was then cleaved by AP endonuclease activity as shown in the figure below.

In support of this mechanism they first provided chemical evidence for the apyrimidinic site at the 3' end of the 5'-labelled fragments. They then purified the enzyme further and were able to separate the glycosylase activity from most (but not all) of the AP endonuclease activity. The glycosylase was specific for DNA containing pyrimidine dimers and the product of the reaction did indeed contain AP sites. Finally, treatment of the product with DNA photolyase or with further exposure to UV (treatments which cleave the cyclobutane ring between the two pyrimidines of the dimer, returning them to their original conformation), resulted in release of free thymine. This could only have arisen from the 'dangling' 5'-pyrimidine of the dimer, formed by the action of the glycosylase.

Similar conclusions have been reached by three groups working with the pyrimidine-dimer specific endonuclease from T4 infected *E. coli*, the product of the T4 *v* (or *den V*) gene. Linn and co-workers (University of California, Berkeley; *Nature* in the press) showed that the product of the reaction of this enzyme with UV-irradiated DNA contained an AP site at the 3'-end of the cleavage site, and Radany and Friedberg (Stanford University; *Nature*, in the press), like Haseltine *et al.* demonstrated that subsequent to enzymatic action by the T4 endonuclease thymine could be released by photoreversal. This release of free thymine was correlated with loss of pyrimidine dimers from the DNA, and could not be effected with extracts of cells infected with *v*-gene mutants of T4.



Seawell, Smith and Ganesan have also studied the action of the T4 UV-endonuclease (*J. Virol.* in the press). Their enzyme preparation contained both glycosylase and AP endonuclease activity, but only the former was active at 0°C. Thus the product of the reaction at 0°C, using colEI-DNA as substrate, contained AP sites, which were only converted into nicks if the temperature was subsequently raised to 37°C. Attempts to separate the two activities by physical procedures were unsuccessful. On the other hand assays of extracts of *E. coli* infected with T4 containing temperature-sensitive mutations in the *v* gene revealed that the glycosylase, but not the AP endonuclease activity, was thermolabile. This shows that the *v* gene codes for the dimer-specific glycosylase activity.

These studies provide convincing evidence that the *M. luteus* and T4 'UV-endonucleases' first rupture the glycosylic bond of the 5'-pyrimidine of the dimer and then cleave the phosphodiester backbone between the resulting apyrimidinic site and

the 3' pyrimidine of the dimer. What is not yet established is whether the glycosylase has intrinsic AP-endonuclease activity or whether the two activities can be separated.

With these two enzymes the distinction between base and nucleotide excision repair has become less clear-cut. We now await results with enzymes from *E. coli* and human cells. In *E. coli* the incision step is controlled by three genes (*uvr A*, *B* and *C*) which code for high molecular weight proteins. Unlike the enzymes from *M. luteus* and T4, the *uvr* gene products recognise other bulky adducts as well as pyrimidine dimers, and the incision step is carried out by a complex of the three gene products in an ATP-dependent reaction (Seeberg, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2569; 1978). Neither the *uvrA* nor the *uvrB* gene product contains glycosylase activity (Seeberg, Norwegian Defense Research Establishment, Kjeller, Norway) and

preliminary results from several groups suggest that the *uvr* gene products do not act via a glycosylase-AP endonuclease sequence, but rather via a classical endonuclease action.

The *uvr* genes have been cloned in several laboratories (Seeberg, Norway; Rupp, Yale University; van Sluis, Leiden); and the properties of the gene products are under intensive study. We can thus expect the elucidation of the mechanism of 'UV-endonuclease' action in *E. coli* within a short space of time.

In human cells the incision step is controlled by at least seven gene products, the seven complementation groups of the genetic disorder xeroderma pigmentosum, all of which are deficient in effecting incision of their DNA after UV-irradiation of the cells. Studies on the enzymes from mammalian cells are, however, fraught with all the worst problems of enzymology (small quantities and extremely labile enzymes), and it may be some years yet before we can explain the mysteries of excision-repair in human cells. □

A.R. Lehmann is in the MRC Cell Mutation Unit, University of Sussex, Brighton.

Prothoracicotrophic hormone-secreting cells in the insect brain

from Lynn M. Riddiford

THE specific cells that produce prothoracicotrophic hormone (PTTH) have been identified in the insect brain (Agui *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 5694; 1979) and this has been followed by identification of the neurohaemal storage organs (see *Nature* this issue, p669). The hormone causes the prothoracic glands to secrete ecdysone which controls the growth and metamorphosis of the insect.

Ablation studies in a variety of insects had attributed PTTH activity to neurosecretory cells in the dorsum of the brain but it was a new, very sensitive assay, developed in Gilbert's laboratory (Bollenbacher *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 5148; 1979) that allowed the identification of the specific cells.

Using isolated prothoracic glands of freshly ecdysed *Manduca sexta* (the tobacco hornworm) pupae and a radioimmunoassay for the secreted ecdysone, they showed that homogenates of larval brains would stimulate the basal rate of ecdysone secretion fourfold. Moreover, half-maximal activation of the glands required only 0.16 brain equivalents. Pupal brains showed similar activity and had the additional advantage that both groups of neurosecretory cells (the medial and the lateral) were visible in the living brain due to iridescence caused by the Tyndall effect. Agui *et al.* first showed that the region of the pupal brain containing the lateral neurosecretory cells had the major portion of the PTTH activity, thus confirming the earlier bioassay results of Gibbs & Riddiford (*J. Exp. Biol.* **66**, 255; 1978). By

an extremely delicate dissection using an eyebrow hair, Agui *et al.* separated the two histologically distinct groups of lateral neurosecretory cells, then isolated the two cells (each about 20 µm diameter) of the most lateral group that had contained the PTTH activity. Although these two cells could not be distinguished morphologically or topographically, only one of them contained the activity of the whole cluster. Thus in the *Manduca* pupal brain only two cells seem to produce PTTH — one in either hemisphere.

The absence of significant PTTH activity in the medial neurosecretory cell cluster was unexpected. In the blood-sucking bug *Rhodnius prolixus* both electrical and secretory activity of the medial neurosecretory cells has been correlated with the time of PTTH release (Steel in *Comparative Endocrinology* (eds. P. J. Gaillard & H. H. Boer) Elsevier, North Holland, 1978). Moreover, in another moth species *Hyalophora cecropia* both groups of cells seem to be necessary for the initiation of the molt from the pupa to the adult (Williams *Symp. Soc. Dev. Biol.* **8**, 61; 1948; Van der Kloot *Amer. Zool.* **1**, 3; 1961). The role of each of these cell groups can now be resolved using the new PTTH assay.

Agui *et al.* also showed that the corpora allata (the endocrine glands that produce juvenile hormone in the larva and in the adult) are the neurohaemal storage organs

for PTTH. Classically the insect corpora cardiaca have been considered to be the repository for the neurosecretory products from the brain. The axon of the PTTH-secreting cell crosses over and exits from the contralateral side of the brain, then passes through the corpus cardiacum to the corpus allatum (Nijhout *Int. J. Insect Morphol. Embryol.* **4**, 529; 1975). Both the pupal and the larval corpora allata contain 4-5 times the PTTH activity of the corpora cardiaca (Agui *et al.*, *Nature* this issue p669). Extraction of the corpora allatal homogenates with hexane to remove the juvenile hormone showed that the stimulation of ecdysone secretion was not due to this hormone. These data strongly support the conclusion that the corpora allata of Lepidoptera are the neurohaemal organs for PTTH, a suggestion first made by Ichikawa and Nishiitsutsuji-Uwo (*Biol. Bull.* **116**, 89; 1959). Whether the corpora allata play a similar role in other insects remains to be seen.

The identification of the cell producing PTTH as well of the neurohaemal storage organ and release site for the hormone paves the way for the isolation and identification of this all-important hormone for insect growth and development. Studies to date suggest that it is a peptide of 5,000 daltons although larger sizes have also been reported (reviewed in Gilbert *et al. Ann. Rev. Physiol.* **42**, 493; 1980). These past studies have used brains as a source of PTTH, but in light of these recent results the pupal corpora allata which seem to store PTTH but are devoid

Lynn M. Riddiford is Professor of Zoology, University of Washington, Seattle.

of allatotropin should be a better source. Small amounts of released hormone for confirmatory studies can probably be obtained from these glands by stimulation of the nerve leading to them or by high K^+ depolarization as has been done with the diuretic hormone of *Rhodnius* (Maddrell &

Gee, *J. Exp. Biol.* **61**, 115; 1974). Also the identification of the PTTH-secreting cell makes possible a detailed neurophysiological study of the control of the release of this hormone by various internal and external signals such as larval size, photoperiod, and temperature. □

Dielectric relaxation spectroscopy

from J. H. Calderwood

If the permittivity (or refractive index) of a dielectric material is measured over a frequency range extending from very low values up to the infrared, a number of absorptions will be observed. Frequencies at which these absorptions occur, and their strengths, may be a decisive factor in determining the practical use to which the materials can be put. Study of the spectra can yield information about the structure of the molecules of the material, and of the molecular dynamics of the system which the molecules, and in some case aggregates of molecules, comprise. These studies and their applications in science and industry were the theme of a recent conference.*

The interpretation of the dielectric measurements presents further problems. H.A. Price of the University College of Wales, Aberystwyth, pointed out that although isotropic liquids usually show only one absorption maximum in their relaxation spectrum, it may be broader than the absorption which would be predicted by the Debye equation. In some cases, the broadening is sufficient to allow the relaxation to be resolved into multiple processes corresponding to whole molecule rotation and the internal rotation of a flexible group within the molecule. Debye considered spherical molecules, but if his treatment is generalised to ellipsoidal molecules as was done by Perrin, then separate relaxation processes are predicted corresponding to rotation about the different molecular axes. Price gave details of the observation of multiple relaxation processes for some liquid crystals, and the particular cases of p-methoxy-benzylidene-p-n-butylaniline (MBBA) and n-heptyl-cyanobiphenyl (7CB).

R. Toomer from the University College of North Wales, Bangor, was concerned with the separation of conduction and relaxation processes as energy loss in a dielectric can be caused by either of them. Metal electrodes are normally used for dielectric measurements, but as they are capable of injecting charge into dielectric solids, thus initiating a conduction process, it is necessary to separate that conduction from the dielectric relaxation phenomena. Toomer described an experimental technique whereby a material could be polarized by

induction, without any direct metallic contact, and the surface scanned by a sensitive induction probe, again without contact being made, to determine the time decay of the surface potential, and so determine the relaxation time of the specimen.

Another technique, especially suitable for the investigation of charge storage mechanisms, is that of field-induced thermally stimulated currents (FITSC). It involves raising of the temperature of a specimen at a known rate while the current is measured. Vanderschueren of the University of Liège gave a critical account of how such measurements should be interpreted and paid particular attention to the tests that should be carried out to ensure that spurious effects are absent. In particular, a systematic variation of experimental parameters which might affect the results should be carried out: these include such factors as electrode material, specimen thickness and field intensity. Other techniques involving thermal relaxation should also be used to confirm the results of FITSC.

The more traditional method of the examination of the frequency spectrum can be used to investigate the behaviour of aggregates of molecules of the micelle type. R. Pottel of the University of Göttingen described measurements in the frequency range 0.1 MHz to 60 GHz on liquid crystalline aqueous systems and showed how the dynamical behaviour could be influenced by the molecules of the solvent, and by the solute concentration. Molecular dynamics can also be studied by the rather unusual technique of non-linear dielectric spectroscopy, when materials are polarized by fields of high strength. If the polarization is expressed by a power series, the second and higher order of coefficients characterise the non-linear properties. Helleman of the University of Leuven described how the non-linear effects observed for reactive systems are an indication of the extent to which the field has perturbed the chemical composition of the sample. Thus from the relaxation of the non-linear effect, information on chemical rates can be collected. This is a very unusual tool to be employed for the investigation of chemical rate processes.

A portion of the frequency spectrum which is more difficult than most is the

millimetre wave region. At longer wavelengths, waveguides, lines, closed cavities, and bridges are available, while at shorter wavelengths optical techniques become applicable. A. Gebbie and P. R. Mason of Imperial College, London, described how over-moded cavities could be used in the millimetre region. The over-moding is produced by means of a rotating paddle in the cavity which scatters the radiation in all directions, thus ensuring that the specimen will be subjected to its influence, without the possibility of its being affected by a special wave pattern. The open resonator provides another very useful means of measurement in the microwave region, and its advantages become particularly significant as the wavelength decreases. A. L. Cullen of University College, London, described the construction of an open resonator suitable for measuring the permittivity of a material in the form of a sheet. In order to assess the error which might result from some small departure from the ideal in a design feature, the interesting technique was adopted of deliberately making the departure large, and making measurements as it was progressively reduced. A. C. Lynch, also of University College, discussed the limitation in the accuracy of permittivity measurements and showed how some of the difficulties might be overcome. For example, in a microwave resonator it is possible to arrange that the surface of the sheet specimen are in a field which is nearly zero, thus reducing the effect of any error in making the most difficult measurement associated with the experiment. Surprisingly, that is the measurement of the thickness of the specimen!

Even when we know how to obtain good results, and how to interpret them, the problem remains of how to predict them. Prediction is made possible by the construction of models which corresponded in some analogous way with the physical reality. Although in principle the construction of such models might be fairly straight forward the analysis of the model to determine the behaviour which it predicts is usually a matter of considerable technical complexity. The problems associated with modelling dielectric behaviour were discussed by M. W. Evans of the University College of Wales, Aberystwyth, P. Bordewijk of Leiden University, and C. Brot of the University of Nice. Computer simulation is liable to be very expensive in computer time, but Brot showed how reliable values of complex permittivity of highly polar fluids could be obtained by a two-dimensional simulation involving only a few hundred molecules, despite the long-range character of dipolar interaction. This is an encouraging advance in a field in which progress is not easy. □

J. H. Calderwood is Professor of Engineering at the University of Salford.

*The conference on 'Dielectric relaxation spectroscopy in Science and Engineering' was held at the Paul Langevin Centre, Aussois, France in March, 1980.



How selfish is DNA?

In the 17 April issue, Doolittle and Sapienza and Orgel and Crick separately used the term 'Selfish DNA' to describe certain DNA sequences in eukaryotic organisms. They argued that the process of DNA replication allows the accumulation within the replicating genome of DNA sequences which have no functional (phenotypic) significance but whose presence stimulates the further accumulation of sequences of the same kind. This 'selfish' DNA is supposed, in particular, to account for some of the apparently functionless DNA in the genomes of higher organisms. The two articles have stimulated a great deal of comment, some of which appears below. The original authors will reply at a later stage.

from T. Cavalier-Smith

WHILE it may be futile to look for a 'special function for every piece of DNA', it is a mistake to imply^{1,2} that selection within the genome for 'selfish DNA' (intragenomic selection) explains the C-value paradox³. One should also not neglect existing evidence for the idea that the overall amount of DNA in the genome has definite (nucleotypic^{3,5-7}), effects on cellular and organismal phenotypes, which are of profound adaptive significance^{3,7}. Orgel and Crick¹ imply and Doolittle and Sapienza² explicitly suggest that the strong and widespread correlations between C-values, cell and nuclear volumes and cell cycle length^{3,5} can be explained simply in terms of an evolutionary equilibrium between a universal, constant, tendency for C-values to be increased by the multiplication of selfish DNA sequences, and selection against such increases, which would be more intense in smaller and more rapidly reproducing cells.

There are two objections to their treatment of the problem. First, even on their theory the decisive factor accounting for the unexpectedly great variation in C-values is variation in the selective force against high C-values. This selective force acts not within the genome, but between genomes differing in C-value i.e. different nucleotypes⁵ (nucleotypic selection). Second, their assumption that nucleotypic selection is always for smaller genomes would only be reasonable if there was no evidence that the overall amount of DNA exerts a causal nucleotypic control on cell and nuclear volume and cell cycle length.

In some circumstances nucleotypic selection for larger genomes is also to be expected³ if causal nucleotypic effects genuinely exist. Two phenomena strongly argue that they do: (1) the increased cell volumes and cell cycle lengths caused by increased numbers of B-chromosomes⁸, (2) the reduction in cell volume caused by chromosome elimination and chromatin diminution³, which occurs in certain invertebrates. These effects are of key importance for our understanding of the C-value paradox, for they provide positive evidence for a differential selective

advantage for different C-values at different stages of the life cycle. As very large amounts of DNA are eliminated in somatic cells, but retained in the germ line, not only must a low C-value be positively advantageous in somatic cells, but also a markedly higher C-value must be of positive selective advantage to the animal in the germ line. The idea that the extra DNA is retained merely because it cannot be got rid of^{1,2} is completely untenable in this case, for a mechanism of elimination clearly exists.

A 'relatively small proportion' of extra DNA would not be strongly selected against, but the extra non-coding DNA whose presence we have to explain is often not merely 'a relatively small proportion' of the total DNA. In single-celled eukaryote algae, C-values vary at least several thousand-fold³, so the amount of non-coding DNA in the highest C-value species is probably many thousands of times that of the coding DNA. Even by Orgel and Crick's own criterion this makes an explanation of the C-value paradox in terms of selfish DNA highly implausible. It seems more reasonable to suppose that large cells actually require more DNA than do small ones.

Whatever the mechanism of evolution of larger cell size, larger cells require more rRNA transport to the cytoplasm per cell cycle than do smaller cells. One would therefore expect selection to increase the rate of RNA transport in larger cells, lest it become rate limiting to cell growth and unduly lengthen the cell cycle. This could be done by increasing the amount of skeletal DNA (S-DNA)³ so as to increase the nuclear surface area and the number of nuclear pores. The suggestion is therefore that excess DNA is used, not, as has been incorrectly stated¹, to slow development but rather to prevent the excessive slowing of development which would otherwise be caused by large increases in cell size³.

It is entirely beside the point that cells can¹ slow their growth without increasing

their genome size, for only if growth is slowed as a secondary consequence of increased cell size (which in practice is invariably associated with increased C-values) does one expect slow growth to be associated with a high C-value³. It is well known that some slow growing organisms have small cells and low C-values. But what should never occur on my theory³ — and has not been observed — is that high C-value organisms have small cells and short life cycles.

Though there are undoubtedly several ways of evolving larger cells^{3,7}, a simple and direct way would be by increasing the number of replicon origins involved in the volume-dependent control of DNA replication^{3,7}. This cannot provide the fundamental explanation of the C-value paradox, since it does not necessitate a larger genome (as it involves only a small fraction of the genome), but it would facilitate the coordinated evolution of cell and nuclear volumes, which the nucleotypic theory³ postulates. It also explains the variation in cell size caused by B-chromosomes and chromatin elimination. On this theory^{3,7} it is the extra replicon origins not the larger nucleus, or the larger genome as such, which increases cell size: what the C-value controls more directly is nuclear volume.

It also seems premature to dismiss all middle repetitive DNA as selfish DNA². Where C-values have greatly increased relatively recently much of it may be selfish in the restricted sense of having no sequence-specific function (but not in the wider sense¹ of lacking nucleotypic effects on the phenotype), as suggested³ for *Plethodon* and *Lathyrus*; but in organisms like mammals with evolutionarily stable C-values it seems to be strongly conserved¹⁰, and there is recent evidence that it may form replicon origins¹¹, as proposed earlier³. Even in *Plethodon* a fraction of middle repetitive DNA is strongly conserved¹² which suggests that there are in general two quite distinct categories of middle repetitive DNA, one with sequence specific functions (and therefore G-DNA³), and the other being a

T. Cavalier-Smith is in the Department of Biophysics, King's College, London.

form of S-DNA having only nucleotypic functions and whose related sequences merely reflect their relatively recent common origin by duplication and transposition and which will eventually by mutation and random drift become unique DNA.

Unless the idea that genome size has a directly causal nucleotypic effect on cell and nuclear volumes and cell cycle length is refuted, it is merely begging the question to speak of the extra DNA in high C-value organisms as 'junk' or having 'no specific function, or 'useless', or having 'no phenotypic expression'. Quantitative scaling⁶ of cell or nuclear volumes and developmental rates is a very specific function, even if it is mediated by DNA the bulk of whose sequences (but not amount) could be changed without changing the phenotype. The idea that nuclear surface area and the number of nuclear pores are functionally important aspects of the phenotype³ is supported by the fact that they are developmentally adjusted (for example in oocytes) to varying cell volumes and rates of transcription: one should not simply ignore evidence that the total amount of DNA, as well as its degree of folding or unfolding, causally influences nuclear surface area.

Intragenomic selection is clearly important for understanding evolution within genomes and for understanding the significance of certain DNA sequences¹⁻⁴, but it does not in itself clarify the evolutionary forces determining genome size; the situation is analogous to that in animal populations where the selective forces acting between competitors tell us little or nothing about what determines the overall population size. Present evidence for direct nucleotypic effects of genome size on cell and nuclear volume and cell cycle lengths suggests that selection acting on these important evolutionary parameters^{3,7} would greatly outweigh selfish-DNA-like effects in determining genome size. But the relevant level of selection here is that acting between genomes of different sizes (i.e. nucleotypic selection), rather than the lower-level intragenomic selection.

As far as evolution of genome size is concerned, the activities of 'selfish' DNA

sequences are best regarded as mutational processes serving to provide the variation in genome size on which nucleotypic selection then acts.

Intragenomic selection may often lead to a slight upward 'mutation pressure' on genome size, but the implication^{1,2} that such pressures are always upwards is unfounded for this would prevent evolutionary reductions in genome size, which seem often to have occurred³. Using the analogy mentioned above, one may say that nucleotypic selection sets the 'population size' of the DNA sequences (i.e. the genome size) within which 'selfish DNA' sequences may compete with each other by intragenomic selection. It is important when discussing genome evolution to distinguish these two levels of selection, both from each other and from the more usual form of selection between alternative alleles, which has been the stock in trade of population geneticists and which it would be better to call allelic selection instead of genic¹³ selection.

This distinction between allelic, intragenomic, and nucleotypic selection enables one to discuss the C-value paradox more precisely than the superficially seductive phrases 'selfish gene' (which in Dawkins' use¹⁴ conflated all three types of selection) or 'selfish DNA' which conflates intragenomic and nucleotypic selection. The term 'intragenomic selection' is clearer than 'non-phenotypic selection'² because it directs attention to the fundamental phenomenon in question — intragenomic competition between different sequences — without begging the question as to the phenotypic effects of the competing sequences, or misleadingly implying that there is a well-defined contrasting concept of phenotypic selection or 'phenotypic benefit'.

The term 'phenotype' as used in the definition of selfish DNA¹, in 'non-phenotypic' selection², and in my earlier discussion of intragenomic selection³, departs radically from the traditional meaning of phenotype¹⁵⁻¹⁷ as embracing all observable traits including such things as transposability of DNA sequences, the renaturation rates of middle repetitive DNA, nuclear volumes or C-values. Since this new restricted use of 'phenotype' to exclude certain traits (but which?) associated very directly with DNA has never been clearly defined, the concepts of 'non-phenotypic selection' and 'selfish DNA' themselves lack precision. It might be better to redefine selfish DNA as DNA whose presence makes no positive contribution to the fitness of the cell or whole organism, and which spreads by intragenomic selection.

Finally, one should emphasise that, though the phrases 'selfish DNA' and 'intragenomic selection' are both new, the concepts they stand for were very clearly formulated 35 years ago by Östergren⁷, who speculated that B-chromosomes were useless parasites.

Ignorant DNA?

from Gabriel Dover

It has recently been suggested that the more the organisation of the eukaryote genome is unravelled the more it can be taken as a depository of many inconsequential acts of DNA replication and transposition. Selfish DNA that has 'subverted the mechanism of duplication'¹ and 'evolved a strategy for survival'² has entered the ordered house of the genes and created mayhem: a state of disorganisation that is of no concern to the organism. Or has it? It is in answer to this question that two recent articles^{1,2} have attempted to review the evidence. Both emphasize, with some qualification, that the majority of the DNA of the eukaryotic genome may indeed be selfish. In doing this they have rightly underscored the molecular and population pitfalls inherent in all naive attempts to define functions for an heterogeneous assemblage of non-coding DNA, which is excessive in mass, and which is organised mainly into families of multiple copies that intervene between and within the genes. This is a refreshing approach and one that has not been over-stressed in the past. However, it is one thing to brush aside the naive functionalist looking for precise molecular interactions and another inadvertently to throw out the baby (that small amount of suggestive evidence) with the bathwater and to replace it with genetically awkward concepts concerning the process of accumulation of 'junk'.

Recent studies of fluctuations in size and position of shared families of sequences in carefully compared genomes of related species³⁻⁶ and of alterations in genomic position of sequences in recently separated populations⁷⁻⁹, have shown that the composition of the higher genome is constantly changing and often mobile²⁵. These changes are occurring both on an evolutionary timescale and within the space of a few generations. The underlying mechanisms are not known but the present-day distribution of sequences between species and patterns of arrangements of sequences within a species can be ascribed to quirks of DNA replication that recurrently amplify and intersperse (or transpose) the new with the old families,¹⁰⁻¹². Although not all investigators would agree on the details, the consensus view would be that DNA is rather an ignorant molecule that frequently gets out of hand and is quite capable of generating a multitude of sequence arrangements. In what ways can we expect the results of this sort of flux to affect the biology of organisms? Doolittle and Sapienza, and Orgel and Crick expect it to have very little obvious molecular influence on the phenotype and consequently they focus their attention on the sociobiology of the change itself. It is at

Gabriel Dover is a lecturer in the Department of Genetics, University of Cambridge and a Fellow of King's College, Cambridge, UK.

1. Orgel, L.E. & Crick, F.H.C. *Nature* **284**, 604 (1980).
2. Doolittle, W.F. & Sapienza, C. *Nature* **284**, 601 (1980).
3. Cavalier-Smith, T. *J. Cell Sci.* **34**, 247 (1978).
4. Cavalier-Smith, T. *Nature* **270**, 10 (1978).
5. Bennett, M.D. *Proc. R. Soc. B* **178**, 277 (1971); *Proc. R. Soc. B* **181**, 109 (1972).
6. Bachmann, K., Chambers, K.L. & Price, H.J. *Pl. Syst. Evol. Suppl.* **2**, 41 (1979).
7. Cavalier-Smith, T. *BioSystems* **12**, 43 (1980).
8. Rees, H. & Jones, R.N. *Chromosome Genetics* (Edward Arnold, London, 1977).
9. Östergren, G. *Bot. Notiser* **2**, 157 (1945).
10. Rubin, C.M. *et al. Nature* **284**, 372 (1980).
11. Jelinek, W.R., *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 1398 (1980).
12. Mizuno, S. & MacGregor, H.C., *Chromosoma* **48**, 239 (1974).
13. Williams, G.C. *Adaptation and Natural Selection* (Princeton University Press, 1966).
14. Dawkins, R. *The Selfish Gene* (Oxford University Press, 1976).
15. Whitehouse, H.L.K. *Towards an Understanding of the Mechanism of Heredity* (Arnold, London 1965).
16. Watson, J.D. *Molecular Biology of the Gene* 3rd edn (Benjamin, Menlo Park, California, 1976).
17. Sinnott, E.W., Dunn, L.C. & Dobzhansky, T. *Principles of Genetics*, 4th edn (McGraw-Hill, New York, 1952).

this point that they construct a process of selfishness^{1,2} and non-phenotypic selection² that in its assumptions overlooks some of the details of genome organisation and activity which reveal a somewhat different process of evolution.

The idea of selfishness was originally used by Dawkins¹³ to highlight the increase in frequency of one gene (allele) in a population at the expense of another. As a caricature of evolutionary processes that appear to endow single genes with 'motivation' it is harmless. As a description of the process of natural selection and adaptation operating on collections of interacting genes locked within genomes, and that may contribute only in part to the selected phenotype, it is inadequate and misleading. Nevertheless, selfish increase in numbers at another's expense could be used to describe the outcome (but not necessarily the process) of any self-replicating unit. If we ignore the unfortunate teleological implications of selfishness we can predict that extant families of DNA would represent a limited range of accumulated sequences that contained within them a high content of highly efficient and mobile replicators. However, with respect to this, there is no obvious molecular commonality in the internal sequence organisation of the many families that have been examined. These families are complex and are of every possible size (number of copies), periodicity ('kinetic complexity'), sequence composition, internal organisation and interspersal. Examples are too numerous to list but recent reports of sequence organisation in rye, cow and fruit-fly reveal the very different intricate patchworks of sequences that have evolved^{4,14,15}.

Considering the data *in toto*, we cannot escape the notion that the genome is subjected to a range of random processes that are capable of generating any conceivable pattern of organisation. Thus there is a clear predictive distinction of the outcome between selfish and stochastic processes. In addition, if the majority of the families have no effect on the phenotype and are also not 'parasitic' (*sensu* Orgel and Crick) then they are simply neutral and spread (or not), according to statistical processes of drift, similar to neutral alleles. It could be that dispersal throughout a sexually reproducing population is accelerated, as Orgel and Crick have rightly suggested, by the fact that copies of a sequence become distributed throughout a karyotype (parasitic DNA) and it is to this particular property, although seemingly common to most of the randomly produced families, that the appellation of selfish could be used if we so wished. There is also the interesting suggestion that diminution and loss of a family are occurring at a much slower rate. However, there would probably be more thrust to the central and important argument that much of the genome makes no direct molecular sense if Doolittle,

Sapienza, Orgel and Crick had clearly underlined the limitless ways of creating and disseminating 'junk' by random processes rather than appearing to rely on the more limited idea of the genome as an arena for selfish replicators under 'non-phenotypic selection'. This is a small but necessary quibble, for in making this distinction a second point of clarification can be raised concerning the biological consequences of these processes.

Selfish DNA, by definition, makes no specific contribution to the phenotype¹ and no phenotypic explanation is required for its continued existence². The attribution of selfishness to the sequences appears to prejudge the issue of their biological effects, whereas judgement of the biological consequences of randomly accumulated sequences is a separate consideration.

Indeed it could be argued that the present levels of accumulation of sequences are due to phenotypic selection for tolerance to extant families rather than to their selfishness. If this were not so one highly efficient replicator might be expected eventually to replace the whole genome. However setting this aside, are we yet in a position, in our ignorance of the mechanisms of so many fundamental biological processes, to strip the bulk of these sequences (whether random or selfish) of all biological activity? To answer this, we need to turn to several diverse and suggestive observations.

First, the overall growth of the genome is not a completely haphazard phenomenon in that it is known from several species groups that chromosome arms increase proportionately in length as species accumulate more DNA, e.g. in salamanders¹⁶. This might be a reflection of some process, perhaps involved in primary transcription or the meiotic behaviour of chromosomes, that requires that certain regions of the genome should be kept as separable and unique entities. If this is so, then there would be a high premium for straightforward selection for a balanced

interspersal of new families of DNA as an aspect of chromosome phenotype.

Second, there are interesting experiments of Davidson and Britten and their colleagues on the activity of sea urchin genomes. They have shown that up to 30% of the genome is transcribed into heterogeneous nuclear RNA, some of which is composed of contiguous stretches of repetitive and structural gene sequences. Very recently they have shown that there are developmental and tissue-specific differences in the abundance of nuclear transcripts of different repetitive families, but not of the structural genes, and that the nuclear RNA falls into many sets, each of which can be characterized by a particular pattern of repetitive sequences¹². Repetitive sequences have been found to be intimately interspersed within developmentally regulated globin gene clusters in rabbit¹⁷ and man¹⁸ which in the latter are homologous to several nuclear RNAs¹⁹. If the splicing and transport of short coding transcripts are affected by differences in organization of the longer transcripts then phenotypic selection might arbitrate between some patterns of dispersion based on this activity. It is also interesting to hear that the very abundant (satellite) DNAs are transcribed during meiosis in new oocytes²⁰.

Third, there is an intriguing correlation, discussed by Orgel and Crick, between total DNA amount (C-value) and the durations of cell cycles and generation time of a species²¹; critical adaptive parameters through which selection might influence the size of a genome.

Fourth, some families of DNA, because of their presence and position, rather than their precise sequence, affect the state of chromatin condensation which in turn can alter both the frequency and localisation of recombination²², and the expression of nearby genes, (position effect variegation). Recently, a correlation has been found between the transposition of sequences and viability²³ and gene activation²⁴ in *Drosophila* and several other species²⁵.

Finally there is evidence that in primates, Gramineae and rodents, specific and quite intricate patterns are shared between chromosomes and between species^{4,5,6,26}. In species of rodents in particular the sharing extends to 'segmental sequence variants' (minor patterns) and their proportions within a family of repeats, despite the evidence that within each species and within each chromosome there are recurrent rounds of amplification^{6,26}. The reasons for the detailed preservation of such patterns are not known and it might be interpreted in terms of selfish sequences. However, we have recently obtained evidence (Dover & Ribbert) that in the blow-fly, all genomic sequences co-replicate during the polytenisation of chromosomes in germline cells but not in polytene somatic cells in which satellite DNA families suffer a preferential under-replication. There

1. Orgel & Crick *Nature* **284**, 604 (1980).
2. Doolittle & Sapienza *Nature* **284**, 601 (1980).
3. Moore *et al. Cell* **15**, 189 (1978).
4. Bedbrook *et al. Cell* **19**, 545 (1980).
5. Donehower & Gillespie *J. Mol. Biol.* **134**, 805 (1979).
6. Brown & Dover *Nature* **285**, 47 (1980).
7. Young *Proc. natn. Acad. Sci. U.S.A.* **76**, 6274 (1979).
8. Strobel *et al. Cell* **17**, 424 (1979).
9. Cameron *et al. Cell* **16**, 739 (1979).
10. Dover *Nature* **272**, 123 (1978).
11. Flavell *et al. in Genome Organisation & Expression in Plants*. (Plenum, 1980).
12. Davidson & Britten *Science* **204**, 1052 (1979).
13. Dawkins *The Selfish Gene* (Oxford UP, 1976).
14. Pech *et al. Cell* **18**, 883 (1979).
15. Wensink *et al. Cell* **18**, 883 (1979).
16. Mizuno *et al. Chromosoma* **88**, 239 (1974).
17. Shen & Maniatis *Cell* **19**, 379 (1980).
18. Fritsch *et al. Cell* **19**, 939 (1980).
19. Jelinek *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 1398 (1980).
20. Varley *et al. Nature* **283**, 686 (1980).
21. Bennett *Proc. Roy. Soc. B* **181**, 109 (1972).
22. Yamamoto & Miklos *Chromosoma* **66**, 71 (1978).
23. Gvozdev *et al. Cold Spring Harbor Symp. XLV* (1980).
24. Gehring & Green *Cold Spring Harbor Symp. XLV* (1980).
25. Movable genetic elements *Cold Spring Harbor Symp. XLV* (1980).
26. Brown & Dover *Nucleic acid. Res.* **8**, 781 (1980).
27. Goldschmidt *Material Basis of Evolution*. (Yale UP, 1940).
28. Dover in *Insect Cytogenetics. Roy. Ent. Soc. Symp.* **10**, (Blackwell, 1980).
29. Stanley *Macroevolution* (Freeman, 1980).

might be a specific need for the retention of all sequence patterns during germ-line differentiation and meiosis.

These varied aspects of organisation and activity suggest, albeit tenuously, that there is some overall selective marshalling of arbitrary sets of sequences which give some specific and common form to closely related genomes. Although the sequences concerned may not intrinsically define a specific function, selection may act on the phenotypic effects arising from the mere presence or position of the sequences within a genome.

When we enter the depths of the higher genome we should not abandon all hope of arriving at an understanding of the manner in which some sequences might affect the biology of organisms in completely novel and somewhat unconventional ways. It was imaginatively suggested by Goldschmidt in 1940²⁷ that saltatory steps in chromosome repatterning lead to abrupt changes in phenotype, some of which ('hopeful monsters') might have a degree of success in particular environments. It may be that the rapid changes in sequence organisation (in particular of the germ-line) can give rise to interesting discontinuities in morphology or developmental timing in such a process of speciation²⁸. Evidence is accumulating that speciation is rarely the result of a gradual and large accumulation of adaptive allelic differences, but that it is episodic and rapid followed by long periods of morphological and genetic constancy, (aptly reviewed in detail by Stanley.²⁹) The accidental and sudden accumulation of extraneous DNA sequences might lead to the inception of a process whose subsequent constancy reflects the much slower rate of diminution and loss of these elements, (among other things). □

Occam's razor

from Temple F. Smith

DOOLITTLE and Sapienza argue that since the evolution of properties such as transposability ensure sequence survival, no other selective or functional properties are required to explain the existence of much of this 'extra DNA'. Orgel and Crick argue, in addition, that for the eukaryote no phenotypic selective pressures of sufficient strength are known to forbid the internal genomic evolution of these nonviral parasitic DNAs. These arguments are at their base a variant on Occam's razor, and have an analogue in the axiom of high energy physics: what (state transition) is not forbidden is mandatory. These apparent solid arguments are supported by considerable data: the large variation in total DNA content among organisms of equal complexity, if not always of close

taxonomic association; and, the recent work on the yeast Ty-1 element (Cameron, Loh & Davis *Cell* **16**, 739; 1979) which is strongly suggestive of just such a parasitic sequence.

No rigorous calculations, however, have been made of phenotypic selections on such extra DNAs. The coordinate evolution required between parasitic sequences and the enzymes manipulating nucleic acids, particularly those with sequence specificity, requires more detailed analysis. There is a need for careful statistical analysis on transposables as to their probability of disrupting functional sequences or on the stability of non-functional repeats generated via such mechanisms as consecutive uneven crossovers. There are also other curious statistics which must be investigated in connection with these ideas. For example, the entire GC content of the vertebrates appears constrained by the genetic code (Smith *Math. Biosci.* **4**, 1979; 1969) to a very narrow range about 42% while invertebrates are less constrained and prokaryotes not at all. This, at first glance, seems in direct contradiction to the expectation of the 'Selfish DNA' hypothesis.

For many evolutionary biologists, there is another problem inherent in these discussions on selfish DNA. It is epistemological in nature, as the presented theory appears nearly irrefutable. If a phenotypic constraint or function is found for any given sequence, it is either removed from consideration under the theory or it is argued that its function was a later adaptation exploiting the already existing parasitic sequence. Similar considerations have, of course, plagued the theory of evolution from its inception. Yet the important question is not to prove the potential for neutral parasitic sequences (of that there seems little doubt) but rather to predict by the 'selfish hypothesis', under known phenotypic gene level selection pressures, the distribution and other statistical characteristics of particular genetic sequences. The more constraining the predictions, the more seriously we must take the theory. □

Selfish DNA in 'Petite' mutants

from R. A. Reid

DIRECT experimental evidence of DNA arising in eukaryotes by non-phenotypic selection is furnished by 'petite' mutations of the mitochondrial DNA of *Saccharomyces cerevisiae*. These mutants are promising systems for investigating the questions raised by Orgel and Crick¹ on the molecular mechanisms involved in the appearance and maintenance of seemingly

superfluous DNA and the selective disadvantages of carrying such DNA. In brief, suppressive 'petite' mutants have defective mitochondria that cannot respire because of the deletion of large segments of mit DNA. Typically, these deletions, which probably arise through site-specific illegitimate recombination events², are accompanied by amplification of the non-deleted DNA so that the total amount of DNA in defective mitochondria is very similar to that of the wild type³. This amplification confers no obvious advantages; the resulting mit genome appears to be irrelevant to the immediate needs of the cell which, being deprived of functional mitochondria, uses energy from sugar glycolysis for growth. In other words spontaneous or artificially induced 'petite' mutations constitute systems where selfish DNA is created before our eyes and confirm that strategies exist for increasing the probability of survival of DNA that does not contribute to organismic phenotypic fitness.

Yeast mit DNA is probably more closely allied to eukaryotic nuclear DNA than to prokaryotic DNA as it contains highly reiterated short sequences and introns^{4,5}. Therefore its small genome size (26 μ) and the ease with which amplification can be induced and identified make it an attractive system for testing models of how middle repetitive and highly reiterated sequences can arise in eukaryotes without phenotypic selection. Progress has already been made in defining the nucleotide sequences that delimit the repeat units of recently arisen and old petite mutants relative to their wild type parental strains⁶. The mit DNA of 'petites' can amount to over 10% of the total cell DNA. Far from contributing to fitness this implies a selective disadvantage of about 10^{-2} if some reasonable assumptions are made about the energy costs, and suggests that under substrate limiting conditions the redundant mit DNA could be substantially eliminated within some thousands of generations. Theoretically it would seem easier for a yeast strain under metabolic stress to rid itself of irrelevant DNA in redundant mitochondria than to carve out selfish DNA already integrated into the nuclear genome, and quite different mechanisms may apply. Nevertheless, detailed long-term studies of the evolution of the mit DNA of new 'petites' grown under defined selective pressures should at least illuminate the proposition that the DNA loads of extant cells represent compromises between the expansionist tendencies of selfish DNA and the restraining forces of selective disadvantage. □

1. Orgel, L.E. & Crick, F.H.C. *Nature* **284**, 604 (1980).
2. Bernardi, G., Prunell, A. & Kopecka, H. In *Molecular Biology of Nucleocytoplasmic Relationships* p85 (ed. Puiseux-Dao, S.) Elsevier (1975).
3. Locker, J., Rabinowitz, M. & Getz, G.S. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1366 (1974).
4. Slonimski, P.P. *et al.* in *Biochemistry and Genetics of Yeasts* p.339 (eds. Bacila, M., Horecker, B.L. & Stoppani, A.O.M.) Elsevier-North Holland. (1978).
5. Bos, J.L. *et al.* *Nature* **275**, 336 (1978).
6. Faugeron-Fonty, G. *et al.* *J. Mol. Biol.* **134**, 493 (1979).

Temple F. Smith is Professor of Physics in the Biophysics Laboratory, Northern Michigan University.

R. A. Reid is in the Department of Biology, University of York, York.

ARTICLES

Th and U in sedimentary rocks: crustal evolution and sedimentary recycling

Scott M. McLennan & S. R. Taylor

Research School of Earth Sciences, Australian National University, Canberra, ACT 2600, Australia

Thorium and uranium abundances in sedimentary rocks increase at the Archean-Proterozoic boundary, in response to an episodic change in the composition of the upper continental crust. Uranium decreases and Th/U increases during post-Archean time due to significant recycling of sedimentary rocks.

SECULAR variations in sedimentary rock compositions are of major importance because they constrain models of crustal evolution and the amount of sedimentary recycling through geological time. (For reviews of major element trends see refs 1–5). Of the important trends noted for the rare earth elements (REE)^{6–11} the most significant is the sharp change in sedimentary REE patterns associated with the Archean-Proterozoic boundary about 2,500 Myr ago. This has been interpreted as being the result of a major episodic change in the composition of the exposed crust at the end of the Archean Era^{9–11}.

Such changes in crustal composition through time (mafic to felsic) should be reflected by a change in Th and U abundances in sedimentary rocks. The possible role of sedimentary recycling can also be assessed from such data. During rock weathering, U⁴⁺ is readily oxidized to U⁶⁺ and forms the highly soluble species [UO₂]²⁺; on the other hand, Th⁴⁺ does not change oxidation state during weathering and remains relatively very insoluble. Hence, if recycling of material is important in the chemical evolution of sedimentary rocks, it should be accompanied by a steady increase in Th/U ratios, as U would be continually lost during successive cycles of weathering and redeposition, providing oxygen is available in the atmosphere-hydrosphere system.

Ronov and Migdisov¹² have examined Th and U distributions as a function of age for sedimentary rocks of the North American and Russian Platforms. Using those data, Veizer^{2,13} noted a general increase, for Th/Al and U/Al, in progressively younger rocks. This has been interpreted as support for a gradual change in the composition of the exposed crust from mafic to felsic^{12,13}. However, the quality of the analytical data, as measured by analyses of international inter-laboratory rock standards, has not been well established in these studies. The trends noted have not been confirmed on other continents. Finally, the Th/Al and U/Al trends could be an artefact of possible decreases in Al in sedimentary rocks⁴.

For several years, this laboratory has been accumulating high quality trace element data, including Th and U, for clastic sedimentary rocks, primarily from Australia, of all ages^{6–8,14–17}. Some 90 samples covering an age range of >3,700 Myr to the present have been analysed. The distribution of Th and U in these rocks, the implications for the evolution of the continental crust, and the possible role of sedimentary recycling are discussed here.

Samples and results

For this type of investigation, the use of fine-grained clastic sedimentary and metasedimentary rock is most appropriate, as

these represent the end product of sedimentary processes. In the present work, we have relied mainly on such rocks, including shales and mudstones of approximately uniform major element composition. In a few places (such as Henbury) where suitable samples were not available, sub-greywackes and siltstones of similar major element composition were used. This is acceptable as these rocks also represent an effective sampling of the upper crust. No significant differences were seen in major element or trace element distributions that could be attributed to the difference in lithology.

Most samples were obtained from drill core material, to minimize trace element mobility due to weathering. Composite samples were avoided because of the possibility of inclusion of aberrant material. Sedimentary rocks which obviously were derived from a first cycle of sedimentation, such as the Devonian volcanogenic greywackes of the Baldwin Formation⁷, were excluded. Samples showing visible or geochemical indications of alteration were also excluded.

All samples were analysed for Th and U by spark source mass spectrography. The details of the method were recently discussed by Taylor and Gorton¹⁸. This technique is sensitive for both Th and U (detection limit 0.02 p.p.m.) and both elements can be accurately determined. Accuracy and precision are better than $\pm 5\%$.

The concentrations of Th and U at the time the rocks were deposited would not be the same as those measured in the rocks today, as both Th and U undergo radioactive decay. Thus the original U concentration would have been more than twice the present measured concentration for a rock deposited 3,800 Myr ago. The Th/U ratio is also affected as the half lives of Th and various uranium isotopes are different. However, the trends noted in the present study are not controlled by such processes as all possible source regions, as well as the samples themselves, have undergone such decay.

The results of this study have been tabulated in two ways. Table 1 summarizes the raw data for Th, U and Th/U, on the basis of location and approximate age. To help identify major trends the data were also grouped into 500-Myr intervals (Table 2). The period 2,000–1,000 Myr was included in one interval due to the lack of samples.

Thorium. The variation of Th in sedimentary rocks with geological time is shown in Fig. 1. During the early Archean (>3,000 Myr) the average abundance of Th was very low, at 1.5 ± 0.8 p.p.m. (errors indicate the 95% confidence interval on the mean). The significance of the very early Archean Th and U data is questionable because of possible metamorphic effects. The other Archean samples have higher Th abundances at 4.5 ± 1.0 p.p.m. There seems to be a sharp increase in Th

Table 1 Summary of Th and U data for sedimentary rocks

Location	Samples (n)	Approximate age (Gyr)	Th*	U*	Th/U*†	Ref.
Godthåb (Akilia association; Greenland)	7	>3.7	1.4 ± 1.4	0.38 ± 0.24	3.1 ± 1.3	17; This study
Godthåb (Malene supracrustals; Greenland)	5	>3.0	1.6 ± 1.5	0.73 ± 0.76	2.4 ± 1.5	This study
Kambalda (Australia)	14	2.8	4.3 ± 1.4	1.3 ± 0.4	3.4 ± 0.3	14
Kalgoorlie (Australia)	7	2.8	4.8 ± 1.4	1.4 ± 0.3	3.4 ± 0.8	7
Hamersley Basin (Australia)	7	2.47	18.1 ± 4.3	3.5 ± 1.2	5.7 ± 2.4	This study
Pine Creek Geosyncline (Australia)	9	2.2	12.8 ± 2.2	3.6 ± 1.2	3.9 ± 0.9	10
Mount Isa (Australia)	5	1.5	11.4 ± 2.9	3.9 ± 3.0	3.5 ± 1.5	6
Henbury (Australia)	3	1.1	14.7 ± 3.4	3.1 ± 1.1	4.8 ± 0.8	15
Amadeus Basin (Pertatataka Formation; Australia)	6	0.85	14.4 ± 2.5	2.6 ± 0.3	5.6 ± 0.7	6
Amadeus Basin (Arumbera Formation; Australia)	3	0.50	9.1 ± 2.8	1.5 ± 0.4	5.9 ± 0.9	6
State Circle (Australia)	7	0.45	14.3 ± 1.1	2.6 ± 0.3	5.5 ± 0.7	6
Perth and Canning Basins (Australia)	5	0.30	16.0 ± 3.3	2.8 ± 0.8	5.8 ± 0.7	6
Perth Basin (Australia)	4	0.22	14.7 ± 13.3	3.1 ± 2.4	4.7 ± 1.8	6
Carnarvon Basin	2	0.15	23.5	3.2	7.6	16

*Uncertainties represent 95% confidence limits on the means.

†Th/U values are the averages of individual Th/U for each sample.

abundances associated with the Archean-Proterozoic boundary (which for these samples is at ~2,500 Myr). Linear regression of the post-Archean Th ungrouped data (Fig. 1) indicates no change through time although this correlation is not statistically significant. The means of the data in the intervals 2,500–2,000 Myr and 500–0 Myr are statistically indistinguishable. We conclude that there is no significant trend in the Th concentrations during the post-Archean. The average concentration of Th in post-Archean samples is 14.6 ± 1.2 p.p.m.

Uranium. Figure 2 displays the grouped U data plotted against geological time. There is a sharp increase in U concentrations similar to that for thorium, associated with the Archean-Proterozoic boundary. The abundance of U seems to decrease during post-Archean times. Linear regression of the U ungrouped data (Fig. 2) seems to confirm such a trend with the correlation significant at ~98% confidence level. A decrease in U during the post-Archean is also indicated by the fact that samples deposited between 2.5 and 1.0 Gyr are statistically distinct from these samples deposited after 1.0 Gyr. The average U concentration in the post-Archean samples is 3.1 ± 0.4 .

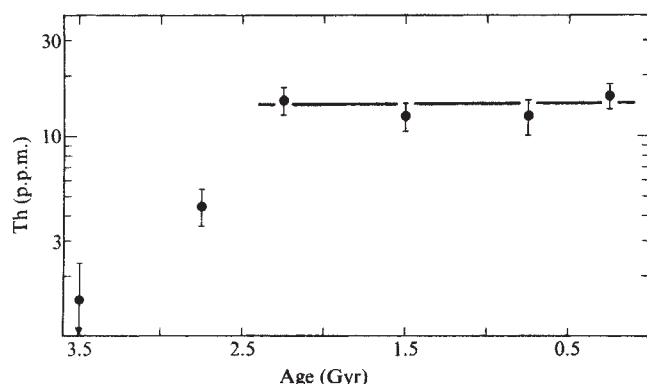


Fig. 1 Plot of Th abundances in sedimentary rocks against geological time. The points and error bars represent the mean and 95% confidence interval, respectively, of the grouped Th data (Table 2). The heavy solid line is based on a linear regression of the post-Archean ungrouped Th data (summarized in Table 1). It is unlikely that Th abundances have changed during post-Archean times.

Th/U ratios. Th/U data are plotted against geological time in Fig. 3. There is a clear trend of increasing Th/U with time for the grouped data. Linear regression of the post-Archean Th/U data illustrates the trend but the correlation is significant only at ~98% confidence level. Inclusion of the Archean data in the regression generates a similar trend (dashed line in Fig. 3) of increasing Th/U with geological time. This correlation is significant at a greater than 99.9% confidence level.

Discussion

The early Archean samples have very much lower Th, U and Th/U ratios than all other samples. If these values are representative of the original concentrations in these rocks, then a progressive increase in Th and U throughout the Archean is suggested. The increasing Th/U ratio through the Archean could imply that sedimentary recycling in an oxidizing environment was an important phenomenon back to >3,700 Myr (see below). Samples used in this study were taken from the Akilia association (>3,700 Myr) and Malene supracrustals (>3,000 Myr) of the Godthåb district in West Greenland. All these rocks have been metamorphosed to amphibolite grade and some may have reached granulite grade metamorphism^{17,19–22}. Recent study of U in metamorphic rocks²³ indicates that this element (and presumably Th as well) is essentially immobile up to amphibolite grade, but is lost, through migrating fluid phases, during granulite metamorphism. Heier²⁴ has also noted the depletion of Th and U in granulites. Studies of early Archean high grade terrains^{24,25} clearly suggest widespread loss of Th and U during high grade metamorphism. The cause of changes in the Th/U ratio during granulite metamorphism is not clear. Although many of the samples used in this study did not reach granulite grade, their proximity to such environments makes the Th and U data suspect and conclusions derived from these data equivocal. Much more work is needed to understand the significance of the low Th, U and Th/U in early Archean sedimentary rocks.

Several recent lines of evidence indicate that the chemical evolution of the upper continental crust is sharply episodic. This was demonstrated by Veizer²⁶ and Veizer and Compston²⁷, who estimated from carbonate rocks, the Sr isotopic evolution of seawater following on the early work of Gast²⁸. The ⁸⁷Sr/⁸⁶Sr ratio of Archean oceans was similar to upper mantle values, but an extremely sharp increase of the radiogenic component ($\Delta^{87}\text{Sr}/^{86}\text{Sr} \sim 0.0025$) was found at about the Archean-Proterozoic boundary. This would

correspond to a major addition of a felsic component to the exposed crust at that time. Studies of REE in sedimentary rocks have also documented a dramatic change in REE patterns associated with the Archean-Proterozoic boundary⁸⁻¹². This was interpreted as a major change in the composition of the upper crust related to the intrusion of large quantities of K-rich granites at the end of the Archean^{9,12}. In addition, the uniform REE pattern in post-Archean sedimentary rocks⁶ indicates that there has been no substantial compositional change during that time.

The Th and U data agree with these models of crustal evolution. Both Th and U abundances show steep increases associated with the Archean-Proterozoic boundary, coinciding with changes shown by Sr-isotopes in carbonates and REE in sedimentary rocks. The intrusion of late Archean K-rich granites into the upper crust could account for this change. Such rocks contain high Th and U concentrations (for example, two late Archean K-rich granites from the Pine Creek Geosyncline have U contents of 3.5 and 5.3 p.p.m. and Th contents of 17 and 21 p.p.m. respectively¹⁰). The lack of any secular variation in Th abundances would also support the contention that there have been no significant changes in upper continental crustal composition during post-Archean times. Plots of Th abundances against time for fine-grained rocks of the North American and Russian Platforms¹² also show no significant change for this element during the post-Archean. Trends noted for U and Th/U ratios are probably a secondary feature and will be discussed below.

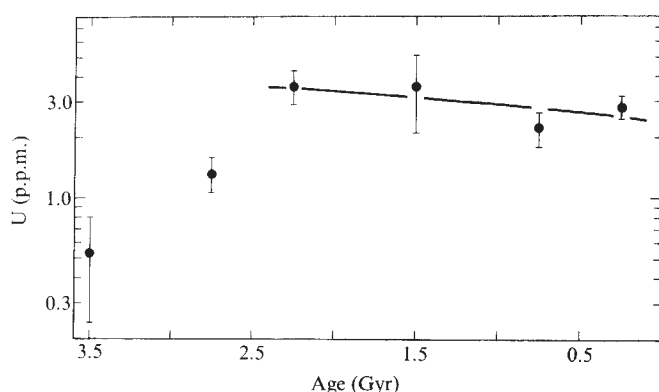


Fig. 2 Plot of U abundances in sedimentary rocks against geological time. The heavy solid line represents a linear regression of the post-Archean ungrouped U data.

It has been pointed out that a suitable test for these models could be made on sedimentary rocks from the late Archean of South Africa (Pongola, Dominion, Witwatersrand Systems)²⁹. These rocks, though clearly Archean in age (>2,700 Myr), closely resemble typical lower Proterozoic sequences. These rocks were deposited after the major crust-forming (intrusive) event in that area. If the models of episodic crustal evolution suggested above are correct, these rocks should show post-Archean geochemical signatures. Five sedimentary samples from the Pongola succession (~3,000 Myr) have been analysed. The results show: Th = 8.9 ± 3.3 p.p.m.; U = 2.7 ± 1.8 p.p.m.; and Th/U = 3.8 ± 1.5 . The Th and U values are significantly higher than those typical of Archean samples and approach values typical for the post-Archean. The Th/U ratio is somewhat low but within the range found for Lower Proterozoic (2.5–2.0 Gyr) sedimentary rocks (average Th/U = 4.7 ± 1.0).

The role of large scale recycling of sedimentary rocks has been discussed by several authors^{1-3,5}. Recently, Veizer and Jansen⁵ have provided detailed models, invoking large-scale sedimentary recycling throughout time, to explain many physical, chemical and isotopic features of the sedimentary record. They proposed that the sedimentary cycle is about 65% cannibalistic.

Table 2 Summary of Th and U data, grouped by age, for sedimentary rocks

Time interval (Gyr)	Samples (n)	Th*	U*	Th/U**
3.0	12	1.5 ± 0.8	0.53 ± 0.29	2.8 ± 0.8
3.0–2.5	21	4.5 ± 1.0	1.3 ± 0.3	3.4 ± 0.3
2.5–2.0	16	15.2 ± 2.4	3.6 ± 0.7	4.7 ± 1.0
2.0–1.0	8	12.6 ± 2.2	3.6 ± 1.6	4.0 ± 0.9
1.0–0.5	9	12.6 ± 2.6	2.2 ± 0.4	5.7 ± 0.5
0.5–0.0	18	15.9 ± 2.3	2.8 ± 0.4	5.6 ± 0.5

*Uncertainties represent 95% confidence limits on the means.

†Th/U values are the averages of the individual Th/U for each sample.

The Th and U data also suggest an important role for sedimentary recycling in controlling the chemical composition of sedimentary rocks, at least as far back as the Archean-Proterozoic boundary. Th and U data should show an increase in Th/U ratio with time if sedimentary recycling is a dominant process. Such a feature is noted in Fig. 3 and seems to be mainly controlled by a decrease in U (Fig. 2), at least during the post-Archean.

The increases in Th/U (and possible decrease in U) in sedimentary rocks, through geological time, is best explained by separation of U from Th due to U loss from repeated cycles of weathering and resedimentation. The sharp changes in the concentrations of Th and U at the Archean-Proterozoic boundary do not easily allow direct extrapolation of this conclusion back into Archean times. However, the average Th/U ratio for Archean samples falls on a trend similar to that delineated by the post-Archean and could suggest that sedimentary recycling in an oxidizing environment has been important well back into the Archean (see further discussion below).

If U is lost selectively from the sedimentary sequences, what is the ultimate fate of this element? Uranium has a much longer residence time than Th and the Th/U ratio of seawater is of the order of 3×10^{-3} (ref. 30). However, the mass of U in the oceans is much lower than in the exposed crust and it is unlikely the oceans themselves could be a major sink. The most likely sink for U is in altered oceanic basalt. Aumento *et al.*³¹ have recorded enrichments of up to a factor of 40 in such rocks. Bloch³² has estimated that between a half and two-thirds of the U delivered to the oceans finds its way into oceanic basalts through hydrothermal alteration or submarine weathering.

The addition of U to oceanic basalt would decrease the Th/U ratios of such rocks. This phenomenon would also result in the subduction of uranium which was derived from the continental crust. The implications of uranium subduction have

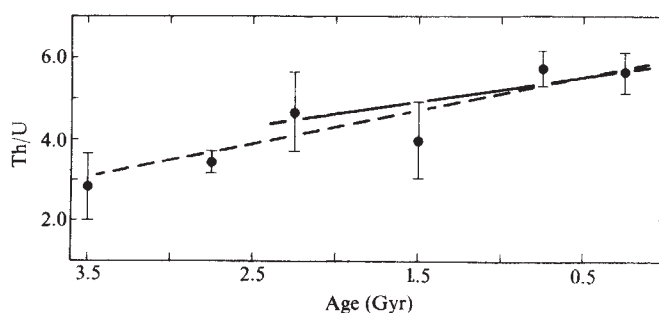


Fig. 3 Plot of Th/U ratios in sedimentary rocks against geological time. The heavy solid line represents a linear regression of the post-Archean ungrouped Th/U data (r is significant at ~98% confidence level). The heavy dashed line represents a linear regression which includes the Archean Th/U data (see Table 3) (r is significant at >99.9% confidence level).

recently been extensively discussed by Fyfe³³. Note that both mid-ocean ridge basalts and island-arc tholeiites have low Th/U. Whether this is due to crustal recycling or to the derivation of both magma types from a mantle depleted in Th relative to U remains questionable.

At least three other controls on the Th and U distributions are possible. Work on Pb-isotope systematics indicates that substantial amounts of U have been lost from many rocks, probably due to weathering^{34,35}. As the Pb-isotopes were not affected in these studies, the weathering must have occurred fairly recently. Samples used in this study were taken mostly from drill core to minimize this effect. We see no reason that a recent episode of weathering should preferentially affect younger rocks, thus producing the U and Th/U trends observed here. On the other hand, the effects of a recent period of weathering, which removes a more or less constant proportion of uranium from all samples, will not affect any of the noted trends.

Many workers have argued that the early Precambrian atmosphere had too little free oxygen to allow efficient solution and mobilization of U during weathering (see, for example, refs 2, 29, 36–39). The best estimate when free O₂ was abundant enough to cause oxidation of uranium is ~2,300 Myr (refs 29, 37). The increase in Th and U abundances at the Archean–Proterozoic boundary cannot be attributed to this feature, as an influx of O₂ would not affect Th and would decrease U in sedimentary rocks. On the other hand, the lack of abundant O₂ during Archean times certainly affects the interpretation of Th/U ratios during this period. The trend of Th/U ratios during post-Archean time demands an oxidizing environment. If the Archean atmosphere was deficient in free O₂, Th/U would not be affected by weathering processes and should remain essentially constant throughout the Archean.

Thus, two models can explain the observed data. First, the Th/U ratio in sedimentary rocks may have steadily increased throughout geological time indicating an oxidizing atmosphere and hydrosphere as far back as about 3,800 Myr. Alternatively, the Th/U ratios of sedimentary rocks may have remained essentially constant throughout the Archean with a substantial change near the Archean–Proterozoic boundary, when Th and U were added to the upper crust (see above and Figs 1 and 2). The Th/U ratio would then steadily increase during the post-Archean due to recycling of sedimentary rocks in an oxidizing environment. Determining the correct model could provide crucial evidence on the nature of the early atmosphere and hydrosphere. Various statistical tests have been performed on the data but are unable to reject either model unequivocally. The question is complicated by the possible metamorphic effects on the early Archean samples (see above). We are initiating a project on early Archean, low

grade metamorphic sedimentary rocks from Western Australia to illuminate this important problem.

Another possible influence on U abundances is the amount of organic carbon in sedimentary rocks. If carbon content has increased with time, this could cause the decreasing U content and increasing Th/U ratio, as U is strongly concentrated in carbon-bearing sedimentary rocks⁴⁰. However, recent studies⁴¹ indicate no clear trends with time for organic productivity and preservation as far back as ~3,400 Myr. Hence it seems unlikely the trends noted in this study are primarily related to secular variations in organic productivity and preservation.

Lower Proterozoic conglomerate-type and unconformity-type U ore deposits are among the largest uranium deposits in the world^{13,42}. These occurrences coincide with the large volumes of U introduced into the upper crust in relation to the intrusion of late Archean K-rich granites. Early dispersion of this material would contribute significantly to the formation of large U-deposits in the Lower Proterozoic.

Conclusions

Th, U and Th/U are very low in early Archean sedimentary rocks compared with sedimentary rocks of all other ages. Whether this is a primary feature or one imposed by metamorphic redistribution is uncertain. A sharp increase in Th and U abundances is associated with the Archean–Proterozoic boundary. This seems to be in response to a dramatic, episodic change in the composition of the upper continental crust related to widespread granitic intrusions at the end of the Archean Era. No evidence for a major change in upper crustal composition during post-Archean times is observed in these data. A secular increase in Th/U and a secular decrease in U is observed in sedimentary rocks during post-Archean time. These features are best explained by sedimentary recycling in the presence of an oxygen-rich atmosphere and hydrosphere, which results in a distinct fractionation of Th and U during successive weathering cycles. Extrapolation of the Th/U trend into Archean times is equivocal. With the present data, models involving gradual changes in the Th/U throughout time or an episodic change in Th/U at the Archean–Proterozoic boundary cannot be distinguished. Rapid dispersion of uranium introduced into the upper crust during the late Archean is probably closely related to the large uranium ore deposits of the Lower Proterozoic.

We thank O. Bavinton, M. Kaye, B. H. Mason, W. Nance, L. Oates, P. Oswald-Sealy and J. M. G. Shelley for assistance with analytical work, Ms Carmel Neagle for assistance in the preparation of this manuscript, the Bureau of Mineral Resources (Canberra), Mt Isa Mines Ltd, O. Bavinton, B. W. Chappell, K. S. Heier, A. Kröner and V. R. McGregor for assistance in collecting or supplying samples and L. Curtis, V. Oversby and J. Veizer for helpful discussions.

Received 26 November 1979; accepted 17 April 1980.

- Garrels, R. M. & Mackenzie, F. T. *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
- Veizer, J. *Contr. Miner. Petrol.* **38**, 261–278 (1973); *Precamb. Res.* **6**, 381–413; *The Origin and Evolution of the Elements* (ed. Ahrens, L. H.) 269–278 (Pergamon, London, 1979).
- Veizer, J. & Garret, D. E. *Precamb. Res.* **6**, 367–380.
- Schwab, F. L. *Geology* **6**, 532–536 (1978).
- Veizer, J. & Jansen, S. L. *J. Geol.* **87**, 341–370 (1979).
- Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **40**, 1539–1551 (1976).
- Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **41**, 225–231 (1977).
- Taylor, S. R. *The Earth: Its Origin, Structure and Evolution* (ed. McElhinny, M. W.) 353–376 (Academic, New York, 1979).
- McLennan, S. M., Fryer, B. J. & Young, G. M. *Geochim. cosmochim. Acta* **43**, 375–388 (1979).
- McLennan, S. M. & Taylor, S. R. *Int. Uranium Symp. Pine Creek Geosyncline* (IAEA, Vienna, in the press).
- Taylor, S. R. & McLennan, S. M. *Precambrian Plate Tectonics* (ed. Kröner, A.) (Elsevier, Amsterdam, in the press); *Phil. Trans. R. Soc. A* (in the press).
- Ronov, A. B. & Migdisov, A. A. *Sedimentology* **16**, 137–185 (1971).
- Veizer, J. *Handbook of Strata-bound and Stratiform Ore Deposits* (ed. Wolf, K. H.) 1–41 (Elsevier, Amsterdam, 1976).
- Bavinton, O. A. & Taylor, S. R. *Geochim. cosmochim. Acta* **44**, 639–648 (1980).
- Taylor, S. R. & McLennan, S. M. *Geochim. cosmochim. Acta* **43**, 1551–1565 (1979).
- Nance, W. B. thesis, Australian National Univ. (1975).
- McGregor, V. R. & Mason, B. H. *Am. Miner.* **62**, 887–904 (1977).
- Taylor, S. R. & Gorton, M. P. *Geochim. cosmochim. Acta* **41**, 1375–1380 (1977).
- McGregor, V. R. *Phil. Trans. R. Soc. A273*, 343–358 (1973).
- Bridgwater, D., McGregor, V. R. & Myers, J. S. *Precamb. Res.* **1**, 179–197 (1974).
- Wells, P. R. A. *Contr. Miner. Petrol.* **56**, 229–242 (1976).
- Bridgwater, D. & McGregor, V. R. *Geol. Unders. Rapp.* **65**, 49–54 (1974).
- Dostal, J. & Capedri, S. *Contr. Miner. Petrol.* **66**, 409–414 (1978).
- Heier, K. S. *Phil. Trans. R. Soc. A273*, 429–442 (1973); *Fortschr. Miner.* **50**, 174–187 (1973).
- Collerson, K. D. & Fryer, B. J. *Contr. Miner. Petrol.* **67**, 151–167 (1978).
- Veizer, J. *The Early History of the Earth* (ed. Windley, B. F.) 569–578 (Wiley, London, 1976).
- Veizer, J. & Compston, W. *Geochim. cosmochim. Acta* **40**, 905–914 (1976).
- Gast, P. W. *Bull. geol. Soc. Am.* **66**, 1449–1454 (1955).
- McLennan, S. M. *Biogeochemistry of Ancient and Modern Environments* (eds Trudinger, P. A. & Walter, M. R.) (Australian Academy of Science and Springer, Berlin, in the press).
- Brewer, P. G. *Chemical Oceanography* Vol. 1, 2nd edn (eds Riley, J. P. & Skirrow, G.) 415–496 (Academic, London, 1975).
- Aumento, F., Mitchell, W. S. & Fratta, M. *Can. Min.* **14**, 269–290 (1976).
- Bloch, S. *Geochim. cosmochim. Acta* **44**, 373–377 (1980).
- Fyfe, W. S. *Chem. Geol.* **23**, 89–114 (1978); *Phil. Trans. R. Soc. A291*, 433–445 (1979).
- Rosholt, J. N., Zartman, R. E. & Nkoma, I. T. *Bull. geol. Soc. Am.* **84**, 989–1002 (1973).
- Oversby, V. M. *Geochim. cosmochim. Acta* **39**, 1107–1125 (1975).
- Cloud, P. *Am. J. Sci.* **272**, 537–548 (1972); *Trans. geol. Soc. S. Afr.* **79** (Annexure) 1–32 (1976).
- Roscoe, S. M. *Huronian Stratigraphy and Sedimentation* (ed. Young, G. M.) 31–47 (Geological Association Canada Special Paper 12, 1973).
- Holland, H. D. *The Early History of the Earth* (ed. Windley, B. F.) 559–567 (Wiley, London, 1976).
- Grandstaff, D. E. *Trans. Geol. Soc. S. Afr.* **77**, 291–294 (1974); *Eos* **55**, 457 (1974).
- Adams, J. S., Osmond, J. K. & Rogers, J. J. *W. Phys. Chem. Earth* **3**, 298–348 (1959).
- Reimer, T. O., Barghoorn, E. S. & Margulis, L. *Precamb. Res.* **9**, 93–104 (1979).
- Dahlkamp, F. J. *Miner. Depos.* **13**, 83–104 (1978).

Energy-dependent uptake of cytoplasmically synthesized polypeptides by chloroplasts

Arthur Grossman, Sue Bartlett & Nam-Hai Chua

The Rockefeller University, New York, New York 10021

Light stimulates the uptake of polypeptides synthesized in vitro into intact chloroplasts. The light-stimulated uptake is inhibited by uncouplers, but not by the electron transport inhibitor dichlorophenyldimethylurea (DCMU) or the protein synthesis inhibitor chloramphenicol (CAP). Addition of ATP to the uptake mixture in the dark mimics the light stimulation of transport, and it reverses the uncoupler inhibition of transport in the light. These data demonstrate that cytoplasmically synthesized polypeptides are imported into the chloroplast by an energy-dependent process.

MANY proteins localized within chloroplasts are synthesized on cytoplasmic ribosomes¹. Such proteins must be transported across the two chloroplast envelope membranes to reach their final destinations in the stroma or thylakoids. Although the precise mechanism of transport is still unknown, recent reconstitution experiments *in vitro* demonstrate convincingly that protein synthesis and transport are independent events² in contrast with co-translational transfer of secretory proteins across the endoplasmic reticulum³.

Synthesis and transport of three major chloroplast proteins have been studied extensively. The small subunit (S) of ribulose-1,5-bisphosphate carboxylase is synthesized *in vitro* as a larger precursor (pS) with an amino terminal chain extension of molecular weight between 4,000 and 5,000 (refs 4–9). If the products of cell-free translation are incubated with intact chloroplasts, pS is taken up, processed to S (refs 6–8) and assembled with endogenous large subunit in the stroma to form the holoenzyme^{6,7}. In addition to pS, larger precursors of two polypeptide constituents, designated 15 and 16, of the light-harvesting chlorophyll *a/b* protein complex have been identified in cell-free translation systems^{10,11}. These precursors (p15 and p16) are also taken up post-translationally by chloroplasts, processed to their mature sizes, and assembled correctly into thylakoid membranes¹¹.

In an attempt to define the mechanism(s) by which cytoplasmically synthesized polypeptides traverse the envelope, we determined experimental conditions for optimal polypeptide transport into intact chloroplasts. In previous studies using suboptimal conditions, light failed to stimulate uptake of pS (refs 6, 7). However, during the present study, we noted that polypeptide uptake by pea chloroplasts is greatly stimulated by light. Here we provide evidence that the light stimulation is due to the production of ATP within the organelle.

Light stimulation of polypeptide uptake by intact chloroplasts

Uptake of many pea poly(A) RNA translation products by intact pea chloroplasts *in vitro* is stimulated by light (Fig. 1). This light stimulation applies to the uptake of stromal proteins as well as polypeptides associated with the thylakoid membranes. Although the extent of the light response varies among experiments, a 2- to 10-fold enhancement of uptake of most proteins is obtained consistently (Fig. 1, Table 1).

Effects of specific inhibitors on polypeptide uptake

Several processes within chloroplasts are stimulated by light including: (1) chloroplast protein synthesis; (2) noncyclic electron flow, cyclic electron flow and the concomitant

production of NADPH and ATP; and (3) the generation of chemical and electrical gradients across the thylakoid membranes and the chloroplast envelope. We used specific inhibitors to determine which of these processes is responsible for the light stimulation of polypeptide uptake. CAP is a specific inhibitor of chloroplast protein synthesis¹⁶. DCMU prevents the formation of NADPH by blocking electron flow between the two photosystems¹⁷. Neither inhibitor alters the light-stimulated uptake of stromal (Fig. 2) or thylakoid membrane polypeptides (results not shown). We conclude from these results that polypeptide uptake by chloroplasts depends neither on concomitant protein synthesis nor on non-cyclic photosynthetic electron flow. As dithiothreitol (DTT) is present in the incubation medium with DCMU, photosystem I would probably be reduced. This would allow for continued cyclic electron flow in the presence of DCMU¹⁸, the generation of chemical and electrical gradients across the thylakoid membranes, the generation of similar gradients across the chloroplast envelope, and the production of ATP. Indeed, protein synthesis in isolated intact chloroplasts is stimulated by DCMU if the incubation medium contains 0.7 mM DTT (unpublished results). Therefore, we examined the effects of uncouplers on polypeptide uptake. Uncoupler effects were assessed quantitatively by excising gel bands

Table 1 Effects of uncouplers and ATP on the uptake of polypeptide A, the small subunit of ribulose-1,5-bisphosphate carboxylase, and polypeptides 15 and 16

Treatment	Radioactivity in excised gel bands (c.p.m.)		
	Poly-peptide A	Small subunit	Polypeptide: 15 and 16
Dark	192	2,495	229
Light	2,619	10,882	1,603
Dark + ATP	2,151	10,178	856
Light + ATP	2,361	10,105	875
Dark + Sal	329	3,165	266
Light + Sal	229	2,167	239
Dark + Sal + ATP	2,890	12,188	964
Light + Sal + ATP	1,906	7,082	504
Dark + SF6847	213	2,364	320
Light + SF6847	376	4,389	242
Dark + SF6847 + ATP	1,768	11,477	736
Light + SF6847 + ATP	1,502	10,054	729

Polypeptide bands were excised from the dried gel and the radioactivity in each band was released by incubation with 20% H₂O₂ (0.5 ml per band) at 50°C for 24 h. The samples were counted in a Beckman LS 8000 after the addition of 10 ml of Aquasol II.

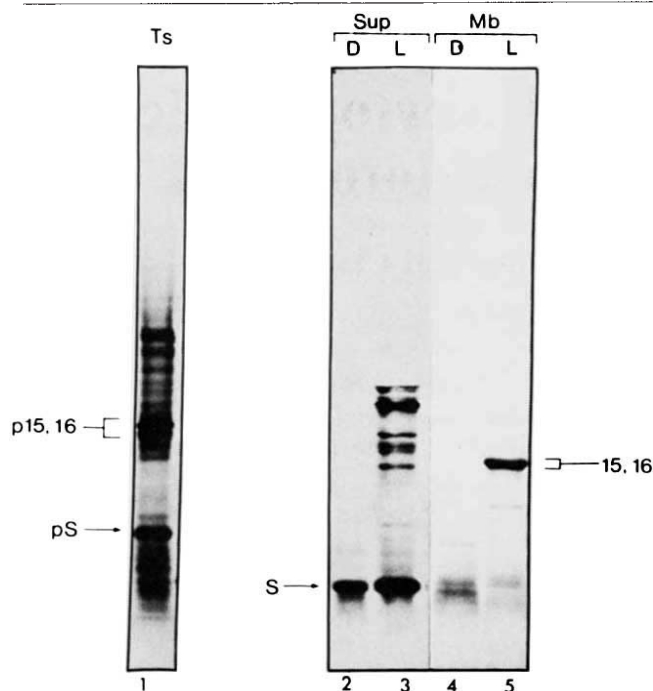


Fig. 1 Effects of light on the uptake of polypeptides translated from pea poly(A) RNA into intact pea chloroplasts. Pea poly(A) RNA was translated in a wheat-germ cell-free system which contained 130 mM K acetate, 1.2 mM Mg acetate, 0.4 mM Tris acetate (pH 7.6), 20 mM HEPES-KOH (pH 7.5), 2 mM DTT, 0.25 mM spermine (neutralized to pH 7.0), 750 $\mu\text{Ci ml}^{-1}$ ^{35}S -methionine ($>600\text{ Ci mmol}^{-1}$), 0.5 mM ATP (neutralized to pH 7.5), 16 μM GTP, 8.4 mM creatine phosphate, 40 $\mu\text{g ml}^{-1}$ phosphocreatine kinase, 24 μM each of the 19 amino acids (minus methionine), 1 absorbance unit per ml of poly(A) RNA, and 0.4 volume of wheat-germ extract¹². After incubation at 27°C for 1.5 h, ribosomes were pelleted (140,000g for 1 h) from the translation mixture and the resulting post-ribosomal supernatant was incubated with intact pea chloroplasts which had been purified on silica sol gradients¹³. The incubation mixture (300 μl) for polypeptide uptake contained 100 μl post-ribosomal supernatant, 200 μg chlorophyll of intact pea chloroplasts, 50 mM HEPES-KOH (pH 8.0), 8.3 mM methionine and 0.33 M sorbitol. After incubation at 25°C for 1 h, the chloroplasts were diluted with 5 ml of 50 mM HEPES-KOH (pH 8.0), 0.33 M sorbitol, pelleted by centrifugation to 4,000g and brake, resuspended in 0.5 ml of HEPES-KOH (pH 8.0), 0.33 M sorbitol and treated with trypsin and chymotrypsin (160 $\mu\text{g ml}^{-1}$ of each) for 30 min at 4°C. Following proteolysis, the chloroplasts were diluted with 5 ml of 50 mM HEPES-KOH (pH 8.0), 0.33 M sorbitol, 1 mM phenylmethylsulphonylfluoride (PMSF), 1 mM Benzamidine-HCl, and 5 mM ϵ -amino-*n*-caproic acid, pelleted, resuspended in 2.0 ml of dilution buffer, and reisolated by centrifugation through a layer of 40% Percoll (3,000g for 3 min; ref. 14). The reisolated chloroplasts were washed with the dilution buffer and lysed in distilled H₂O containing 1 mM PMSF, 1 mM benzamidine-HCl and 5 mM ϵ -amino-*n*-caproic acid. The thylakoid membranes were separated from the stromal proteins by centrifugation at 12,300g for 15 min and both fractions were prepared for electrophoresis. Samples were run on SDS gels containing a 7.5–15% acrylamide concentration gradient^{6,7}. The gels were stained, destained and fluorographed¹⁵. 1, *In vitro* translation products (Ts) of pea poly(A) RNA; 2 and 3, polypeptides imported into chloroplast stroma (Sup) in the dark (D) and light (L) (9,000 lux), respectively; 4 and 5, polypeptides imported into chloroplast thylakoid membranes (Mb) in the dark (D) and light (L) (9,000 lux), respectively. Polypeptides 15 and 16, the constituent polypeptides of the light-harvesting chlorophyll *a/b*-protein complex; p15 and p16, precursors to 15 and 16; S, the small subunit of ribulose-1,5-bisphosphate carboxylase; pS, precursor to S.

corresponding to four polypeptides and assaying their radioactivity. Salicylanilide XIII (Sal, ref. 19) completely abrogates the light-stimulated uptake and processing of pS, p16 and p15, and an unidentified polypeptide whose mature product is designated A (molecular weight $\sim 45,000$). Similarly, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile (SF6847), another uncoupler¹⁹, suppresses the enhanced level of polypeptide uptake in the light but inhibition of uptake of pS is not as complete as that of the other polypeptides (Table 1).

Effects of ATP on polypeptide uptake

Results obtained with the uncouplers indicate that the light stimulation is due either to ATP production directly or to a pH gradient or membrane potential across the thylakoid membrane and/or the chloroplast envelope. As chloroplasts prepared from young pea plants can translocate ATP from the medium into the stroma²⁰, we examined the effects of exogenous ATP on polypeptide uptake. Addition of 10 mM ATP to the incubation medium substantially increases transport of chloroplast polypeptides in the dark (Fig. 3, Table 1). The extent of ATP stimulation differs for stromal and thylakoid membrane polypeptides. While ATP in the dark elevates the transport of stromal polypeptides to the light level (Fig. 3), ATP-stimulated uptake of most thylakoid-membrane polypeptides never achieves the light level (see Table 1 for polypeptides 15 and 16; entire profile not shown). Direct measurements of radioactivity reveal that ATP is as effective as light in stimulating uptake of the precursors to S and

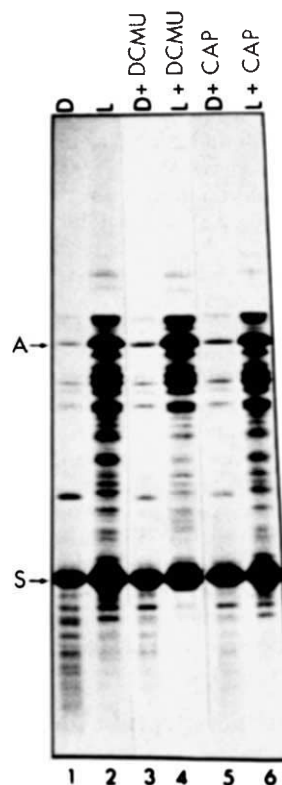


Fig. 2 Effects of DCMU and CAP on the uptake of polypeptides into the chloroplast stroma. *In vitro* reconstitution of polypeptide uptake was performed as described in the legend to Fig. 1. Samples were analysed on SDS gels containing a 12–18% acrylamide gradient and 8 M urea (Matlin and N.-H.C., unpublished method). 1 and 2, Stromal polypeptides imported in the dark (D) and light (L), respectively; 3 and 4, stromal polypeptides imported in the presence of DCMU (2 μM) in the dark and light, respectively; 5 and 6, stromal polypeptides imported in the presence of CAP (100 $\mu\text{g ml}^{-1}$) in the dark and light, respectively. S, the small subunit of ribulose-1,5-bisphosphate carboxylase; A, an unidentified stromal polypeptide of molecular weight $\sim 45,000$.

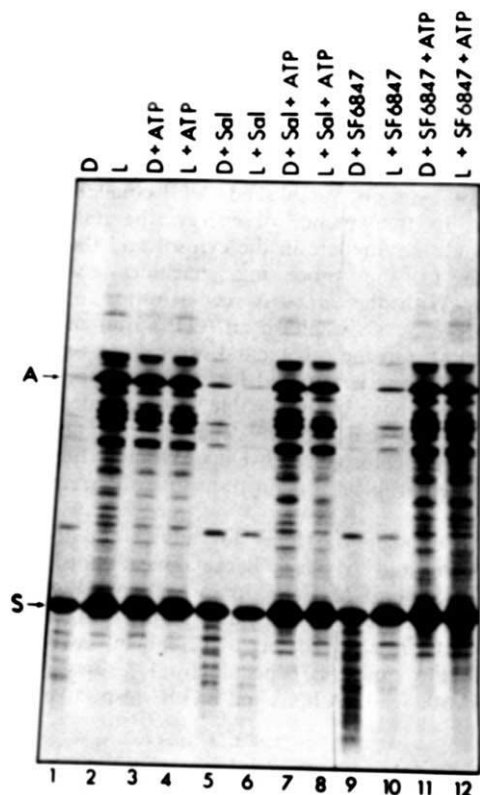


Fig. 3 Effects of uncouplers and ATP on the uptake of polypeptides into the chloroplast stroma. *In vitro* reconstitution of polypeptide uptake was performed as in Fig. 1 and samples were analysed as in Fig. 2. 1 and 2, stromal polypeptides imported in the dark (D) and light (L), respectively; 3 and 4, stromal polypeptides imported in the presence of 10 mM ATP in the dark and light, respectively; 5 and 6, same as 1 and 2, respectively, except with the addition of 2 μ M Sal; 7 and 8, same as 3 and 4, respectively, except with the addition of 2 μ M Sal; 9 and 10, same as 1 and 2, respectively, except with the addition of 2 μ M SF6847; 11 and 12, same as 3 and 4, respectively, except with the addition of SF6847 (2 μ M). S, small subunit of ribulose 1,5-bisphosphate carboxylase; A, unidentified stromal polypeptide.

polypeptide A (Table 1). ATP-stimulated transport of p15 and p16 is approximately fivefold over the dark control; however, the level attained is only 55% of the light level (Table 1). Illumination of chloroplasts in the presence of ATP has little effect on polypeptide uptake, indicating that light and ATP do not act synergistically (Fig. 3, Table 1). Perhaps uptake is limited by some factor other than energy. In these conditions, uptake of p15 and p16 in the light is consistently greater when ATP is not present (Table 1). The reason for this is unclear at present.

While the present results suggest that light-stimulated polypeptide uptake is due to the photoproduction of ATP we could not rule out the possibility that the exogenously added ATP is used inside the chloroplast to generate both chemical and electrical gradients. Because such gradients are collapsed by uncouplers, we examined the effects of Sal and SF6847 on polypeptide uptake sustained by ATP. Sal has no inhibitory effect on the ATP-stimulated polypeptide uptake in the dark, but is slightly inhibitory in the light (Figs 3 and 4; Table 1). The latter effect may be due to increased ATP hydrolysis in the presence of the uncoupler. Similar results were obtained with SF6847, another uncoupler (Fig. 3, Table 1). These results provide conclusive evidence that the stimulation of polypeptide transport by ATP is independent of its ability to generate a chemical or electrical gradient.

Conclusions

We have shown that transport of pea poly(A) RNA translation products into pea chloroplasts *in vitro* is increased severalfold by light. Three lines of evidence demonstrate that the light stimulation is due to the production of ATP by photosynthetic phosphorylation: (1) exogenous ATP mimics light in elevating dark polypeptide transport; (2) light stimulation is inhibited by uncouplers of photosynthetic phosphorylation and (3) the inhibitory effects of uncouplers are reversed by ATP. Taken together, these results also demonstrate that energy in the form of ATP is required to move polypeptides across the chloroplast envelope. Previous studies purporting to show no effect of illumination on uptake of polypeptides by pea chloroplasts were performed in suboptimal conditions^{6,7} in which ATP was probably not the limiting factor.

Barley and Romaine lettuce chloroplasts exhibit light-stimulated uptake similar to that reported here for the pea, while little or no light stimulation is observed with spinach chloroplasts (unpublished results). Spinach chloroplasts contain high levels of endogenous chloroplast ATP even in the dark²². Accordingly, the energy requirement for polypeptide transport into all chloroplasts is probably absolute. The level of dark uptake in our *in vitro* system probably reflects the concentration of endogenous ATP in the organelle plus ATP added exogenously with the translation mix.

This conclusion may seem to be in conflict with data from other studies. For instance, etioplasts and developing plastids which cannot yet synthesize ATP in the light contain polypeptides of cytosolic origin²³. Moreover, mutants of

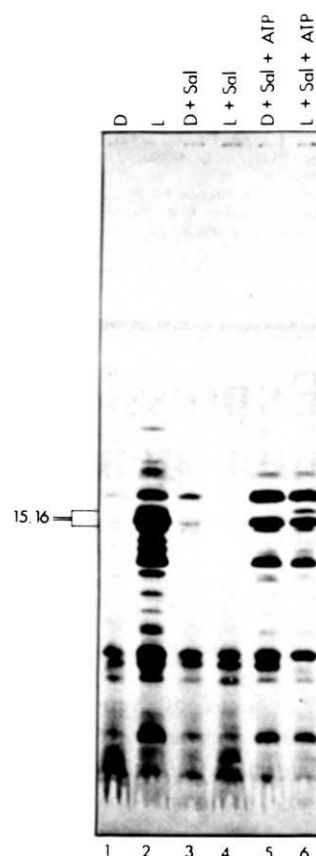


Fig. 4 Effects of Sal and ATP on the uptake of thylakoid membrane polypeptides. The experiments were performed as in Fig. 3. The thylakoid membranes were separated from the chloroplast envelope according to the method of Poincelot and Day²¹. 1 and 2, thylakoid membrane polypeptides imported in the dark (D) and light (L), respectively; 3 and 4, thylakoid membrane polypeptides imported in the presence of 2 μ M Sal in the dark and light, respectively; 5 and 6, same as 3 and 4, respectively, except with the addition of 10 mM ATP.

Chlamydomonas reinhardtii defective in photosynthetic phosphorylation contain thylakoid membrane polypeptides synthesized on cytoplasmic ribosomes²⁴. However, plastids incapable of photosynthetic phosphorylation can recruit ATP in at least three different ways: (1) ATP might be imported directly from the cytosol via the envelope adenine nucleotide translocator²⁵. (2) Indirect energy transfer from the cytosol can be accomplished by the uptake of dihydroxyacetone phosphate, a glycolytic intermediate, through the envelope phosphate carrier²⁶; oxidation of dihydroxyacetone phosphate in the chloroplast stroma would generate ATP; (3) dihydroxyacetone phosphate is also produced within the organelle by phosphorolysis of starch, which occurs commonly in etioplasts and developing chloroplasts²⁶.

Previous studies have established that pS (refs 6-8) and precursors to membrane polypeptides 15 and 16 (ref. 11) are imported into chloroplasts after their synthesis is complete. This post-translational mode of transport has now been confirmed for many as yet unidentified chloroplast proteins localized in the stroma or associated with the thylakoid membranes^{11,12}. The results presented here show that the transport of these chloroplast proteins requires ATP. In contrast, synthesis and transport of secretory proteins are concomitant events. The driving force for membrane traversal is presumably provided by elongation of the growing polypeptide chain and the folding of the nascent chain on the cisternal side of the endoplasmic reticulum membrane.

Received 28 January; accepted 16 April 1980.

- Gillham, N. W., Boynton, J. E. & Chua, N.-H. *Curr. Topics Bioenerg.* **8**, 211-260 (1978).
- Chua, N.-H. & Schmidt, G. W. *J. Cell Biol.* **81**, 461-483 (1979).
- Blöbel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 835-851 (1975).
- Dobberstein, B., Blöbel, G. & Chua, N.-H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1082-1085 (1977).
- Cashmore, A. R., Broadhurst, M. K. & Gray, R. E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 655-659 (1978).
- Chua, N.-H. & Schmidt, G. W. in *Photosynthetic Carbon Assimilation* (eds Siegelman, H. W. & Hind, G.) 325-347 (Plenum, New York, 1978).
- Chua, N.-H. & Schmidt, G. W. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6110-6114 (1978).
- Highfield, P. E. & Ellis, R. J. *Nature* **271**, 420-424 (1978).
- Schmidt, G. W., Devillers-Thiery, A., Desruisseaux, H., Blöbel, G. & Chua, N.-H. *J. Cell Biol.* **83**, 615-622 (1979).
- Apel, K. & Klopstsch, K. *Eur. J. Biochem.* **85**, 581-588 (1978).
- Schmidt, G. W., Bartlett, S., Grossman, A. R., Cashmore, A. R. & Chua, N.-H. in *Genome Organization and Expression in Plants* (ed. Leaver, C. J.) 337-351 (Plenum, New York, 1980).
- Grossman, A. R., Bartlett, S., Schmidt, G. W. & Chua, N.-H. *Ann. N.Y. Acad. Sci.* (in the press).

However, because high energy compounds are required for translation, the exact energy requirement for co-translational transport cannot be resolved.

Nelson and Schatz²⁷ reported that yeast cells depleted of mitochondrial ATP accumulate precursors to certain mitochondrial proteins, demonstrating that energy is required for processing these precursors. As the subcellular location of these precursors was not established, ATP could be required for transport. In the absence of energy, the untransported precursors would accumulate in the cytosol and therefore not be accessible to the processing machinery within the mitochondria. With the *in vitro* reconstitution experiments reported here we clearly establish an ATP requirement at the level of transport. On the other hand, we cannot rule out the possibility that energy is required for precursor processing as well because we have been unable to separate the two phenomena. Joyard and Douce²⁸ characterized an ATPase associated with the chloroplast envelope. Whether this ATPase plays a role in chloroplast protein transport is currently under investigation.

We thank Professor U. Heber for advice and discussion, Linda Bornstein and Michael Becker-Flügel for technical assistance and Richard McCarty for his gift of SF6847. This work was supported by NIH research grants GM-21060 and GM-25114, NIH research career development award GM-00223 to N.-H.C., and NIH postdoctoral fellowships GM-06444 and GM-06678 to A.R.G. and S.G.B., respectively.

- Morgenthaler, J.-J., Marden, M. P. F. & Price, C. A. *Archs Biochem. Biophys.* **168**, 289-301 (1975).
- Mills, W. R. & Joy, K. W. *Planta* **148**, 75-83 (1980).
- Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).
- Ellis, R. J. *Biochim. biophys. Acta* **463**, 185-215 (1977).
- Izawa, S. & Good, N. E. *Meth. Enzym.* **24**, 355-377 (1972).
- Crowther, D., Mills, J. D. & Hind, G. *FEBS Lett.* **98**, 386-390 (1979).
- Heytler, P. G. *Meth. Enzym.* **55**, 462-472 (1979).
- Robinson, S. P. & Wiskich, J. T. *Biochim. biophys. Acta* **461**, 313-340 (1977).
- Poincelot, R. P. & Day, P. L. *Physiol.* **54**, 780-783 (1974).
- Inoue, Y., Kobayashi, Y., Shibata, K. & Heber, U. *Biochim. biophys. Acta* **504**, 142-152 (1978).
- Bradbeer, J. W. in *Biosynthesis and its Control in Plants* (ed. Milborrow, B. V.) 279-302 (Academic, London, 1973).
- Bennoun, P., Masson, A., Piccioni, R. & Chua, N.-H. in *Chloroplast Development* (ed. Akoyunoglou, G.) 721-726 (Elsevier, Amsterdam, 1978).
- Heldt, H. W. *Horiz. Biochem. Biophys.* **2**, 199-229 (1976).
- Heber, U. & Walker, D. A. *Trends biochem. Sci.* **4**, 252-256 (1979).
- Nelson, N. & Schatz, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4365-4369 (1979).
- Joyard, J. & Douce, R. *FEBS Lett.* **51**, 335-340 (1975).

Expression of a chicken chromosomal ovalbumin gene injected into frog oocyte nuclei

M. P. Wickens*, S. Woo†, B. W. O'Malley† & J. B. Gurdon*

*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

†Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Ovalbumin is synthesized by Xenopus oocytes injected with a plasmid containing either the chicken chromosomal ovalbumin gene or a plasmid from which the 5' region of the chromosomal gene has been deleted. However, oocytes injected with a plasmid containing full-length ovalbumin cDNA do not synthesize ovalbumin, despite the fact that at least as much stable, ovalbumin-specific RNA is transcribed from the cDNA as from the chromosomal gene.

THE production of functional mRNA from a eukaryotic gene involves many steps, including transcription, RNA splicing and base modification, transport from the nucleus and assembly into polyribosomes. Previous work has shown that *Xenopus* oocytes can carry out these steps from injected genes¹⁻⁶. Oocytes injected with cloned sea urchin histone genes synthesize functional messengers for histones H2A and H2B (ref. 6). Because the coding sequences of these genes are not interrupted by an intervening sequence, no RNA splicing is

necessary. Oocytes injected with SV40 DNA synthesize the virion proteins VP1, VP3 (ref. 4) and the tumour antigens small t and large T (ref. 5); the production of functional mRNA for the T tumour antigen requires the precise excision of a single intervening sequence⁷⁻⁹.

In the study reported here, we have examined the expression of a cloned chicken ovalbumin gene injected into *Xenopus* oocytes. Our criterion for expression was the synthesis of ovalbumin protein. This assay provides a particularly stringent

test of the RNA processing activities involved in messenger production, because at least six intervening sequences must be removed from the primary transcripts before the RNA can serve as a template for ovalbumin synthesis^{10,11}.

We considered the following problems. (1) Do oocytes injected with the chromosomal ovalbumin gene synthesize ovalbumin? (2) How does the amount of ovalbumin synthesized (after at least six splicing events) compare with the amount of SV40 VP1 synthesis (after not more than one splice)? (3) The cap site and TATATAT sequence at the 5' terminus of the chromosomal gene have been implicated in transcription initiation in chicken oviduct cells; are these regions essential for the production of functional ovalbumin mRNA in oocytes injected with the chromosomal gene? (4) Are any non-structural sequences present in the chromosomal clones necessary for expression; that is, do oocytes injected with a full-length cDNA clone synthesize ovalbumin? We have extended previous experience with injected oocytes by analysing in some detail the expression of a gene which is strikingly regulated in development and whose transcription depends on RNA polymerase II activity.

Ovalbumin DNA templates

The following published information^{10,11} concerning the structure of the chicken ovalbumin gene is relevant. Seven intervening sequences (introns) interrupt the ovalbumin messenger sequence in the chromosomal gene; six of these lie within the protein-coding region. The seventh lies within the 5'-untranslated region of the mRNA sequence, separating the first 45 untranslated nucleotides by 1.6 kilobases from the translation initiation codon at position 65. These first 45 residues have been termed a leader sequence by analogy with eukaryotic viral gene structure^{10,11}.

We examined the expression of three recombinant plasmids containing ovalbumin DNA (Fig. 1). pOV230 is a cDNA clone in which all but the 5'-terminal 12 nucleotides of the

messenger sequence have been copied and inserted into the plasmid pMB9¹². Both pOV8 and pOV12 are clones of the ovalbumin chromosomal gene in pBR322¹³. pOV12 contains 12 kilobases of chicken DNA, including all ovalbumin exons and introns, plus 3.78 kilobases to the 5' side of the cap site and 0.66 kilobases to the 3' side of the polyadenylation site. It contains that A-T-rich region, 32 bases before the cap site, which has been suggested to be involved in transcription

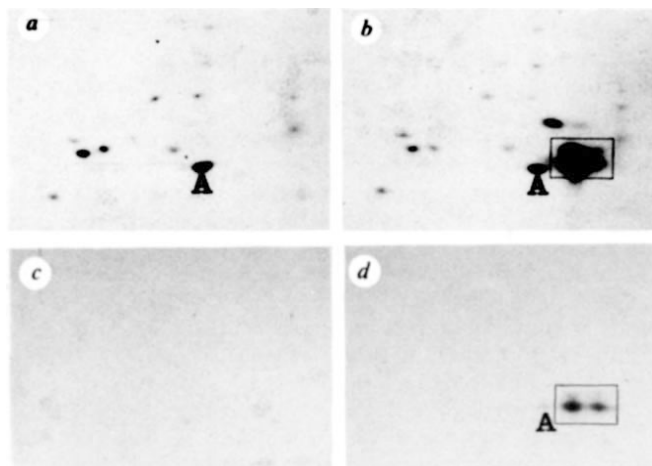


Fig. 2 Position of ovalbumin in two-dimensional polyacrylamide gels of unfractionated oocyte extract and anti-ovalbumin immunoprecipitates. The same central region of each gel is shown. A designates actin; a rectangle surrounds the four ovalbumin spots. The gel is in the same orientation as the gel in Fig. 3. The dark spot to the upper left of ovalbumin in *b* is probably conalbumin, based on its intensity relative to ovalbumin and apparent molecular weight³³. *a, c*, Oocytes injected with buffer only; *b, d*, oocytes injected with 40 ng of chick oviduct poly(A)-containing RNA; *a* and *b*, unfractionated homogenates; *c* and *d*, anti-ovalbumin antibody immunoprecipitates. Polysomal poly(A)-containing RNA from oestrogen-stimulated chicken oviduct was prepared as before³³. Ovalbumin mRNA was about 20% of the total nucleic acid in the sample as judged by denaturing gel electrophoresis³⁴. Oocytes were injected either with injection buffer³⁵ or with 40 ng chick oviduct poly(A)-containing RNA, aiming at the equatorial band. After injection, they were transferred directly into a small volume of MBS culture medium³⁶ (5 μ l per oocyte) containing ³⁵S-methionine (1–2 mCi per oocyte, 1,000–2,000 Ci mmol⁻¹) and incubated for 16–24 h. Oocytes plus their labelling medium were homogenized in 15 mM Tris, pH 6.8, and 150 μ g phenyl methyl sulphonyl fluoride PMSF per ml³⁷, with a final volume of 10–20 μ l per oocyte. Oocyte homogenates were then extracted with an equal volume of trichloroethane (freon) and centrifuged for 15 min at 13,000 g to remove yolk protein. No ovalbumin is lost into the organic phase at this step, as judged by gel electrophoresis of extracted and unextracted samples. Extracted homogenates were re-centrifuged to remove residual cell debris. When necessary, homogenates were stored at –20 °C. An immunoprecipitation reaction mixture of 150–250 μ l was prepared which contained the following: 10⁵–10⁷ acid-insoluble c.p.m. of the ³⁵S-methionine-labelled extract (1–20 oocytes), 10 mM sodium phosphate, 150 mM NaCl, 1% Triton X-100, and 1% sodium deoxycholate³⁸. Anti-ovalbumin antibody was added, and the mixture was incubated for 30 min at room temperature. Unlabelled purified chick ovalbumin (5 μ g) was then added. Incubations were continued at room temperature for 4 h, then transferred to 30 °C for 30 min. Immunoprecipitation reactions were then overlaid onto discontinuous sucrose gradients in soft Brinkmann microfuge tubes, and precipitates were pelleted by centrifugation³⁸. In these conditions, the antibody quantitatively precipitates the 5 μ g ovalbumin carrier (data not shown). Ovalbumin cross-reacting material present in the extract should have bound to the antibody in the first incubation, before the addition of exogenous ovalbumin. Quantitative recovery of cross-reacting material in the extract is ensured by the addition of carrier ovalbumin. To verify experimentally that most ovalbumin in the extracts was recovered, aliquots of an mRNA-injected homogenate were electrophoresed either directly or after immunoprecipitation. As judged by the relative intensities of the ovalbumin spots, recovery was extremely high. Unfractionated extracts were prepared for electrophoresis as described by O'Farrell¹⁶. Immunoprecipitates were prepared as follows. After centrifugation of immunoprecipitation reactions, tubes were frozen on dry ice and cut with a razor blade so as to retain the pellet and a small amount of the sucrose gradient. The sucrose was removed, 30 μ l of 0.1 M NaOH was added and the tubes were incubated at 60 °C for 15 min. The base was then neutralized with 0.1 M HCl, adjusted to 0.1 M Tris, pH 6.8, and brought to 1×A buffer concentrations¹⁶. Solid urea was then added to a final concentration of 9 M. Two-dimensional electrophoresis was carried out as before¹⁶, using a 12.5% polyacrylamide-SDS gel in the second dimension³⁹. Gels were fluorographed⁴⁰, dried and exposed to hypersensitized film.

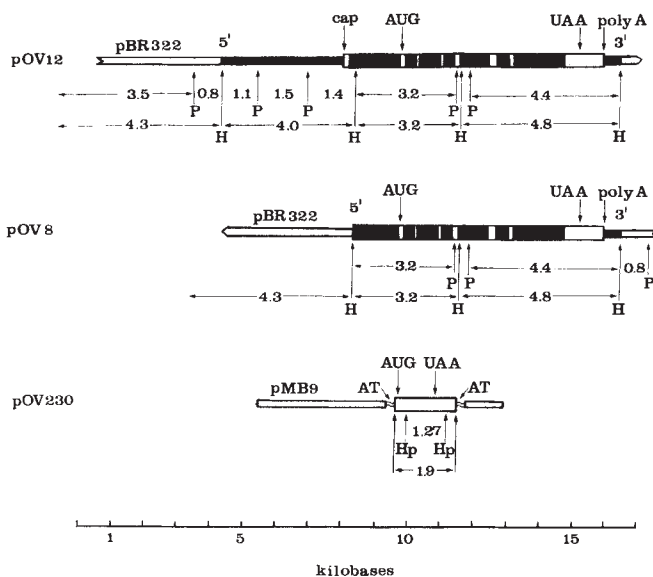


Fig. 1 Structure of cloned chicken ovalbumin genes injected into oocytes. pOV12 and pOV8 are clones of the chromosomal ovalbumin gene; pOV230 is a full-length ovalbumin cDNA clone. Their construction has been described elsewhere^{10,12–14} (detailed structural information in refs 10, 12). pOV12 and pOV8 were constructed by ligation into the *Hind*III site of pBR322; pOV230 was constructed by dA:dT tailing into the *Eco*RI site of pMB9. Note that the chicken DNA in pOV8 and pOV12 is inserted in opposite orientations. Unshaded, thinner regions represent vector sequences; shaded thinner regions, chicken DNA outside the ovalbumin gene (not between the cap site and the polyadenylation site); unshaded thicker regions, exons (coding); shaded, thicker regions, introns (spliced out of the primary transcript). $\approx$, dA:dT tails. The position of the cap site, polyadenylation site, and translation initiation (AUG) and termination (UAA) sites are indicated. All *Hind*III (H) and *Pst*I (P) sites in pOV12 and pOV8 are indicated. The only *Hpa*I (Hp) sites shown are those in pOV230 which are relevant to Fig. 5b.

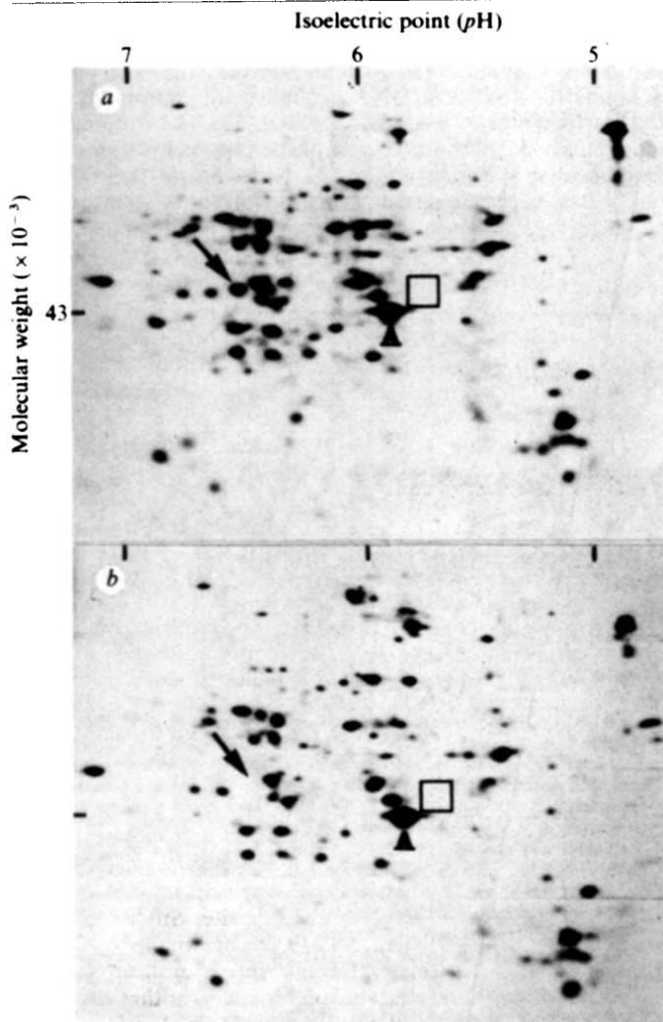


Fig. 3 Two-dimensional gel electrophoresis of unfractionated homogenates of oocytes injected with pOv12 or SV40 DNA. *a*, Oocytes injected with SV40 DNA; *b*, oocytes injected with pOv12. The arrow indicates the position of VP1; the square indicates the position of ovalbumin; A designates actin. 10 ng DNA was injected per oocyte, aiming for the nucleus¹. Groups of about 20 injected oocytes were incubated in a large volume of MBS-H for 36 h, then transferred to fresh MBS-H (5–10 μ l per oocyte) containing ³⁵S-methionine (10 μ Ci per oocyte, 1,000–2,000 Ci mmol⁻¹) for a further 36 h. In these conditions all isotope is taken up by the oocytes; 25–50% is incorporated into acid-insoluble material. All other procedures were as described in Fig. 2 legend, except that a 10% polyacrylamide-SDS gel³⁹ was used in the second dimension.

initiation *in vivo*^{11,14,15}. pOv8 may be regarded as a deletion of pOv12 in which the 5' 4-kilobase *Hind*III fragment has been removed. The chicken DNA is reversed in orientation relative to pBR322.

Ovalbumin is synthesized by oocytes injected with chromosomal gene

In this section we describe a sensitive assay for ovalbumin and demonstrate that injection of the 'complete' chromosomal gene, in pOv12, results in the synthesis of ovalbumin. This expression requires transcription of the chicken DNA, splicing of at least six introns, transport of the mRNA to the cytoplasm, and translation.

In all these studies we assayed ovalbumin production by two-dimensional gel electrophoresis¹⁶ of either unfractionated oocyte homogenates or anti-ovalbumin antibody immunoprecipitates. The second, more sensitive method shows clearly that ovalbumin is produced, while the first aids in quantification.

We determined the location of ovalbumin in these two-dimensional gel assays by using oocytes injected with crude chicken ovalbumin mRNA (Fig. 2). Unfractionated

homogenates of mRNA-injected oocytes display a cluster of four ovalbumin spots absent in saline-injected controls (Fig. 2*a,b*, in rectangle). These lie in the correct molecular weight region for ovalbumin (43,500) and are specifically precipitated by anti-ovalbumin antibody, as demonstrated in Fig. 2*c,d*. (The presence of four prominent spots may in part result from modifications which ovalbumin is known to undergo in chicken cells *in vitro*^{17,18}. The heterogeneity in isoelectric point could reflect the presence and absence of phosphorylation, because oocytes accurately modify proteins they normally do not contain^{19,20}. Oocytes are known to glycosylate ovalbumin²¹, but this probably is not responsible for heterogeneity in the SDS-polyacrylamide dimension (A. Colman, personal communication). Heterogeneity in molecular weight may reflect errors in translation termination in which termination does not occur at the normal UAA codon, but at the in-phase UGA codon 12 bases downstream³⁰.) In these mRNA-injected oocytes, ovalbumin constituted 15% of the total labelled protein; immunoprecipitates from saline-injected controls contained a background of 0.5% of the total. Because the level of ovalbumin is high, no background spots are seen in the gels of immunoprecipitates (Fig. 2*c,d*). If the level of ovalbumin is reduced 100-fold, as we anticipated it might be in oocytes injected with pOv12, the 0.5% background appears as a complex array of spots representing proteins nonspecifically trapped in the immunoprecipitate (data not shown).

The analysis of unfractionated extracts of pOv12-injected oocytes is shown in Fig. 3. The position of ovalbumin is indicated by a square and contains no detectable new spots when pOv12-injected and control SV40-injected oocytes are compared. Virion protein 1 (VP1 position indicated by arrows) is among the most highly labelled spots obtained from oocytes injected with SV40 DNA, in agreement with previous results⁴. We conclude that any ovalbumin made in pOv12-injected oocytes is present at a level considerably less than that of VP1 in SV40-injected oocytes.

The analysis of anti-ovalbumin antibody immunoprecipitates by two-dimensional gel electrophoresis demonstrates that ovalbumin is indeed synthesized in oocytes injected with pOv12 (Fig. 4*a-c*). The background of spots is similar in immunoprecipitates from pBR322 (Fig. 4*b*) and pOv12 (Fig. 4*a*) injected oocytes. Those injected with pOv12, however, produce the four characteristic ovalbumin spots, whereas those injected with pBR322 do not. Moreover, the most basic pair precisely co-migrate with exogenous ovalbumin added to a control immunoprecipitate (Fig. 4*c*).

pOv12-directed synthesis of ovalbumin is eliminated by co-injection of α -amanitin at a concentration (1 μ g ml⁻¹) which inhibits RNA polymerase II but not III (data not shown)^{2,22}. The correct polymerase—polymerase II—therefore is responsible for generating the transcripts which are ultimately translated into ovalbumin.

We quantified the level of ovalbumin in pOv12-injected oocytes by comparison with the level of VP1 in oocytes injected with SV40 DNA. That ovalbumin is present at a relatively lower level is clear from our analysis of unfractionated homogenates (Fig. 3) and from the intensity of the ovalbumin spots compared to those in the background of immunoprecipitates (Fig. 4*a*). We compared by microdensitometry the summed intensity of the four ovalbumin spots in Fig. 4*a* with that of the single prominent VP1 spot (Fig. 3*a*). Ovalbumin is present at 2.1% of the VP1 level by this analysis. (A correction has been made for the relative methionine contents of ovalbumin and VP1 (Fig. 4 legend).) This figure relies on the quantitative recovery of ovalbumin from oocyte homogenates as is apparently achieved by our immunoprecipitation procedure (Fig. 2 legend). VP1 is roughly 0.5% of the total labelled protein in oocytes injected with SV40 DNA⁵; ovalbumin is therefore about 0.01% of the total.

In summary our analysis of oocytes injected with pOv12 demonstrates that ovalbumin is synthesized. We identified the

protein by three independent criteria: isoelectric point, molecular weight and antigenic specificity. Although it is present at only about 2% of the level of VP1 in SV40 DNA-injected oocytes, it is remarkable that any is produced, in view of the number of processing steps required to generate functional ovalbumin messenger. We conclude that frog oocytes can carry out all those steps with a primary transcript of the 'complete' chicken ovalbumin gene.

Ovalbumin synthesis does not require 5' end of chromosomal gene

We next analysed oocytes injected with pOv8 to determine whether the 5' region of the ovalbumin gene, present in pOv12, is essential for expression. As discussed earlier, pOv8 is similar to pOv12 except that the 5' 4 kilobases of the chicken DNA insert have been deleted. It therefore lacks both the cap

site and the TATATAT sequence implicated in transcription initiation *in vivo*^{11,14,15}. pOv8 does, however, retain the second exon intact, and therefore still has the chicken AUG translation initiation codon.

Injection of pOv8 results in the synthesis of ovalbumin at a level similar to that of pOv12 (Fig. 4d). The same characteristic four spots are seen in the proper position, with intensities comparable to those found in immunoprecipitates from pOv12-injected oocytes. The 5' flanking sequence, cap site, leader sequence and first intron can all be deleted without affecting the level of ovalbumin production. We conclude that in *Xenopus* oocytes, none of the steps required to generate functional messenger from a primary transcript of the ovalbumin gene is contingent upon transcription initiation at the sequence thought to be involved in transcription initiation in chicken cells *in vivo*. The expression of pOv8 also suggests that ovalbumin synthesis in pOv12-injected oocytes does not require initiation at or near the cap site in the cloned chicken DNA.

Oocytes injected with full-length cDNA clone synthesize no ovalbumin

Homogenates prepared from oocytes injected with the full-length cDNA clone, pOv230, contain no detectable ovalbumin as assayed by two-dimensional gel electrophoresis of anti-ovalbumin immunoprecipitates (Fig. 4e). This suggests that pOv230 lacks sequences present in the genomic clones that are required for expression, particularly because the amounts of ovalbumin-specific transcripts in oocytes injected with pOv230 and pOv12 are comparable (see below).

Taken together, the expression of pOv8 and non-expression of pOv230 suggests that some genomic sequences are required for the production of functional messenger in injected oocytes, but that they do not lie near the cap site. Studies of SV40 mutants^{23,24} and β -globin: SV40 recombinants^{25,26} have indicated that the splicing of transcripts is critical for their stability and subsequent expression. We suggest that the defect in pOv230 expression relative to the genomic clones also may reflect the absence of intervening sequences.

Level of ovalbumin transcripts in DNA-injected oocytes

The following experiments establish that the lack of ovalbumin synthesis in pOv230-injected oocytes is not due to a lack of transcripts containing ovalbumin messenger sequences. In addition, the data indicate that most stable transcripts from the chromosomal clones are not transcribed exclusively from

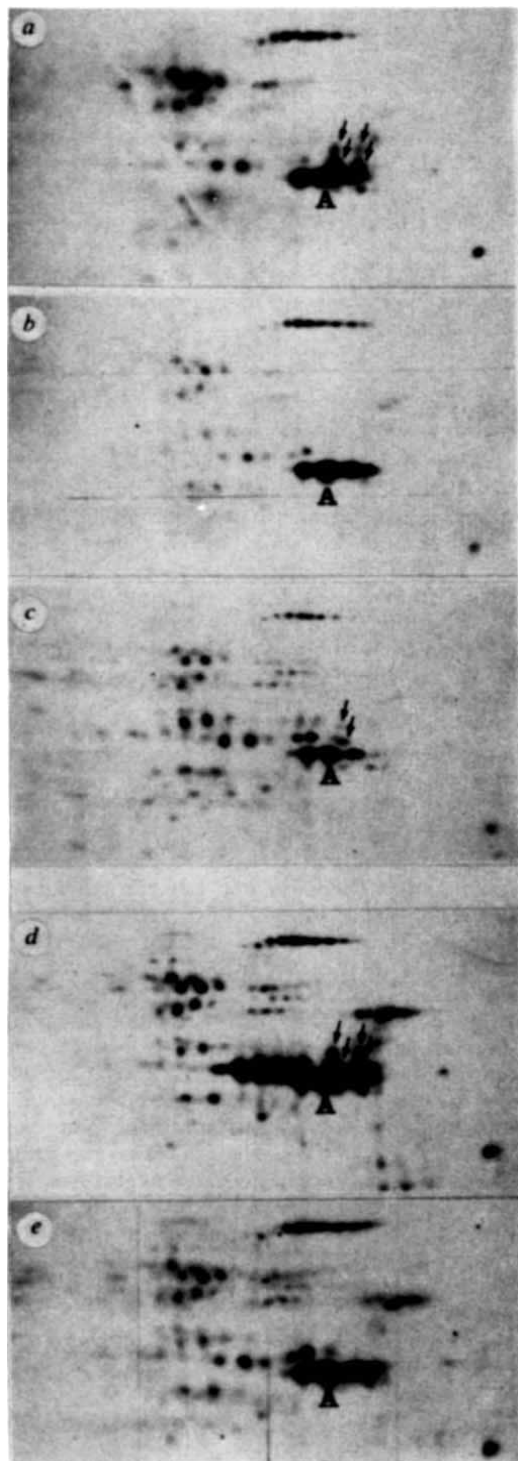


Fig. 4 Anti-ovalbumin immunoprecipitates of DNA-injected oocytes analysed by two-dimensional gel electrophoresis. All manipulations were carried out as described in Fig. 3 legend. The same central region of each gel is shown. Arrows indicate the position of ovalbumin spots; A designates actin. The gel is in the same orientation as the gel in Fig. 3. *a*, Oocytes injected with pOv12; *b*, oocytes injected with pBR322; *c*, same as *b*, but with the addition of a trace amount of ovalbumin mRNA-injected oocyte homogenate (same sample as in Fig. 2b, hence only two of four spots seen); *d*, oocytes injected with pOv8; *e*, oocytes injected with pOv230. In *e* the spot just above and slightly to the right of actin is not ovalbumin but a background spot of variable intensity; it sometimes is obscured by large amounts of ovalbumin (*a* and *d*), but is just visible in *b* and *c* as well as in *e*. In *c* it can be distinguished from the nearest (lower left) ovalbumin spot. The heterogeneity of background spots, in which rows of several tightly packed spots seem to have been derived from a single protein, is ascribed to artefactual charge heterogeneity (see ref. 16). The base treatment involved in sample preparation is likely to destroy certain amino acid residues⁴¹, and result in charge heterogeneity. To quantify ovalbumin expression relative to VP1, the total optical density of the four spots shown in *a* was compared to that of the single VP1 spot shown in Fig. 3a. Fluorograms of shorter exposure were used. Twenty-five times more homogenate was used for the ovalbumin immunoprecipitation than for the VP1 analysis, in order to produce spots of comparable intensity with similar exposures. Optical density was measured using a Joyce-Loebl microdensitometer. Entire spots were scanned. The conditions of film exposure used yield a linear relationship of optical density to radioactivity⁴⁰. Corrections were made for the relative amounts of radioactivity analysed, relative exposure times, and the relative methionine contents of VP1 (2.2%, ref. 9) and ovalbumin (4.4%, ref. 30).

Table 1 Level of stable transcripts synthesized from various injected DNA templates

DNA injected	% Oocyte ³² P-RNA hybridized (d.p.m.)*	% ³ H-cRNA hybridized (d.p.m.)†	% Oocyte ³² P-RNA complementary to injected DNA‡
pBR322	5.3 (9,850)	17.0 (85,000)	31.1 %
pMB9	16.7 (31,050)	78.8 (181,000)	21.2
pOv230	6.1 (11,280)	32.3 (80,700)	18.8
pOv8	2.5 (3,520)	23.9 (59,900)	10.6
pOv12	3.6 (4,970)	19.5 (77,900)	18.4
SV40	6.9 (9,770)	32.3 (129,500)	21.6

Oocytes were injected with 10 ng supercoiled DNA and 0.5 μ Ci α -³²P-rGTP, then incubated at 19 °C for 48 h. Oocyte ³²P-labelled RNA was prepared by homogenizing 20–30 oocytes in 1 ml of 40 mM Tris, pH 7.5, 4 mM EDTA, 0.3 M NaCl, 2.0% SDS and 2 mg proteinase K per ml, extracting the homogenate with an equal volume of phenol:chloroform (1:1), and precipitating with ethanol^{6,42}. ³H-cRNA was synthesized from each template DNA using *E. coli* RNA polymerase with sigma factor (given by A. Travers) and ³H-UTP (52 Ci per mmol, Amersham). A mixture of oocyte ³²P-RNA and ³H-cRNA was redissolved in formamide-PIPES buffer and hybridized to 11-mm diameter DBM-filters on which 50 μ g of DNA had been immobilized²⁷. Hybridizations were incubated for 30 h at 42 °C, the filters were washed, and the hybrids were eluted with 0.4 N NaOH as described²⁷. All hybridizations were performed in duplicate.

*Total ³²P radioactivity in each hybridization ranged from 1.4×10^5 d.p.m. to 2×10^5 d.p.m. Less than 0.2% of each ³²P-labelled or ³H-labelled RNA hybridized to filters bearing calf thymus DNA. These low backgrounds have been subtracted.

†The total ³H radioactivity in each hybridization ranged from 1.5×10^5 c.p.m. to 5×10^5 c.p.m.

‡Per cent of oocyte ³²P-RNA complementary to injected DNA = (% ³²P-RNA hybridized)/(% ³H-RNA hybridized) $\times$ 100. This calculation assumes that the hybridization efficiencies of oocyte ³²P-RNA and ³H-cRNA are identical.

the inserted chicken DNA; rather, most transcription of the chromosomal gene seems likely to result from readthrough from the vector (see discussion).

First, the total amount of stable RNA transcribed from each DNA template was determined. ³²P-labelled RNA prepared from DNA-injected oocytes was hybridized to DBM-paper filters on which large amounts of the injected type of DNA had been immobilized²⁷. Hybridization efficiency was internally monitored in each assay by the addition of ³H-cRNA. Table 1 shows that transcripts from the templates examined constitute from 10 to 30% of the total acid-insoluble radioactivity incorporated by the oocytes, a value consistent with previously reported results¹. Because total acid-insoluble incorporation does not change substantially in oocytes injected with any of these DNAs (data not shown), we conclude that each template produces approximately the same amount of stable RNA. In particular, the quantities of stable RNA transcribed from pOv12 and pOv230 do not differ significantly.

Second, the relative amounts of stable RNA transcribed from the chicken and vector regions of the ovalbumin plasmids were determined. ³²P-labelled RNA prepared from oocytes injected with a cloned ovalbumin DNA template was hybridized to filters to which DNA restriction fragments had been transferred from an agarose gel and immobilized²⁸. (For relevant restriction map data, see Fig. 1.) After hybridization, filters were treated with ribonuclease, and the radioactivity hybridized to each fragment was determined by microdensitometry or scintillation counting.

Transcripts from pOv12: ³²P-labelled RNA prepared from oocytes injected with pOv12 or pOv8 yields similar results when hybridized to the *Hind*III restriction fragments of pOv12 (Fig. 5a1,2). In both cases, three to five times more RNA hybridizes (per kilobase of DNA) to the pBR322 fragment (4.3 kilobases) than to the chicken DNA fragments (3.2, 4.0 and 4.8 kilobases). Hybridization of pOv12 RNA to the *Hind*III–*Pst*I fragments of pOv12 presents the same general pattern, in that the 3.5-kilobase fragment which contains only pBR322 sequences is most highly labelled (Fig. 5a4). Moreover, transcripts of the 1.1- and 1.5-kilobase fragments which flank the gene on the 5' side are present at approximately the same level per kilobase of DNA as transcripts from the 1.4-kilobase fragment which contains the cap site and first exon. These data indicate that most stable transcripts of pOv12 are derived from pBR322, not from the ovalbumin gene.

Transcription of both chicken and vector DNA is strongly inhibited by a concentration of α -amanitin previously

shown^{2,22} to inhibit SV40 and plasmid transcription in oocytes without affecting the transcription of tRNA or 5S RNA genes (Fig. 5a3). Therefore RNA polymerase II is responsible not only for the transcription of those RNAs which are translated into ovalbumin (see above), but also for most of the total RNA complementary to pOv12.

Transcripts from pOv230: Comparison of the levels of ovalbumin-specific RNA in pOv230- and pOv12-injected oocytes is complicated by the fact that pOv230 was constructed by A-T-tailing into the *Eco*RI site of pMB9¹² whereas pOv12 was constructed by ligation into the *Hind*III site of pBR322¹³. To compare the level of transcripts from ovalbumin structural sequences directly, ³²P-labelled RNA from pOv230- and pOv12-injected oocytes was hybridized to filters bearing immobilized *Hph*I fragments of pOv230 (Fig. 5b). The 1.27-kilobase fragment is composed exclusively of sequences found in ovalbumin mRNA (from position 336 to 1,605)³⁰. The 0.85-kilobase fragment is exclusively vector DNA (part of the tetracycline resistance gene common to pMB9 and pBR322 (position 453 to 1,306 in pBR322))⁴³. In pOv230-injected oocytes, the abundance of stable transcripts from the 1.27-kilobase fragment is roughly equal (per kilobase of DNA) to the abundance of stable transcripts from the 0.85-kilobase pMB9 fragment (Fig. 5b 1; see Fig. 5b legend). Moreover, about five times more labelled RNA homologous to the 1.27-kilobase fragment is found in oocytes injected with pOv230 than in oocytes injected with pOv12 (Fig. 5b2; for comparison of pOv230 and pOv12, levels of hybridization to the 1.27-kilobase fragment were standardized relative to the 0.85-kilobase fragment, as detailed in Fig. 5b legend). Although these data suggest that the lack of ovalbumin synthesis in pOv230-injected oocytes is not due to a lack of ovalbumin-specific transcripts, the possibility remained that the dA:dT homopolymeric tracts used to insert the cDNA might act as transcription terminators such that only the nonsense strand of the ovalbumin cDNA insert in pOv230 was transcribed. This was not the case, however, because slightly more pOv230 RNA hybridizes to the sense strand than to the nonsense strand of the 1.27-kilobase *Hph*I pOv230 fragment (Fig. 5c). Because transcripts of the ovalbumin region of pOv230 require no splicing events to juxtapose adjacent exons, it seems likely that the concentration of RNA molecules possessing the contiguous messenger sequence is, in fact, higher in pOv230-injected oocytes than in those injected with pOv12. Thus the lack of ovalbumin synthesis in oocytes injected with pOv230 is not correlated with a lack of transcripts containing the necessary structural information.

Discussion

The ability of oocytes injected with the 'complete' chromosomal ovalbumin gene, pOv12, to synthesize ovalbumin, clearly demonstrates that frog oocytes can perform all the steps necessary to generate functional mRNA from initial transcripts of this gene. In particular, the production of functional ovalbumin messenger requires that at least six introns present in a precursor RNA be accurately removed by RNA splicing.

An 'incomplete' ovalbumin gene that lacks the leader sequence, cap site, TATATAT region and 5' flanking sequence, directs ovalbumin synthesis with about the same efficiency as the intact gene. Therefore, at least when the ovalbumin gene is present as part of a recombinant plasmid, none of these regions is absolutely required for transcription, accurate RNA processing, transport or translation. These results do not necessarily show that the same regions have no function in normal ovalbumin gene expression in the chicken oviduct.

Our data demonstrate that a functional mRNA can be generated from a primary transcript lacking the proper 5' terminus. This raises an intriguing possibility, particularly because we suspect that most ovalbumin transcripts are derived from transcription which 'reads through' from the vector (see below). Perhaps the normal production of

functional mRNA from some genes does not require initiation of transcription at a precise sequence.

Oocytes injected with a full-length cDNA clone synthesize no detectable ovalbumin, yet accumulate more stable RNA containing ovalbumin messenger sequences than do oocytes injected with the chromosomal gene. The deficiency of functional mRNA among pOv230 transcripts may be due to the absence of introns. It has been suggested from work in other systems that RNA splicing has a critical role in the production of functional mRNA²³⁻²⁶. Our results with pOv230 are consistent with this proposal. In these other systems, however, splicing seems to be necessary for the stabilization of primary transcripts^{21,22,26}; apparently this is not the case with transcripts from the ovalbumin region of the cDNA clone, because they are, in fact, somewhat more abundant than those from the ovalbumin region of the chromosomal clone.

There are several possible explanations for our finding that the amount of ovalbumin produced by oocytes injected with the chromosomal gene is roughly 2% of the amount of VP1 produced by oocytes injected with SV40. One concerns the difference in the number of processing steps required to generate VP1 and ovalbumin messengers. The ovalbumin gene contains seven introns and the VP1 gene only one. If splicing occurs with 50% efficiency for each intron, then ovalbumin

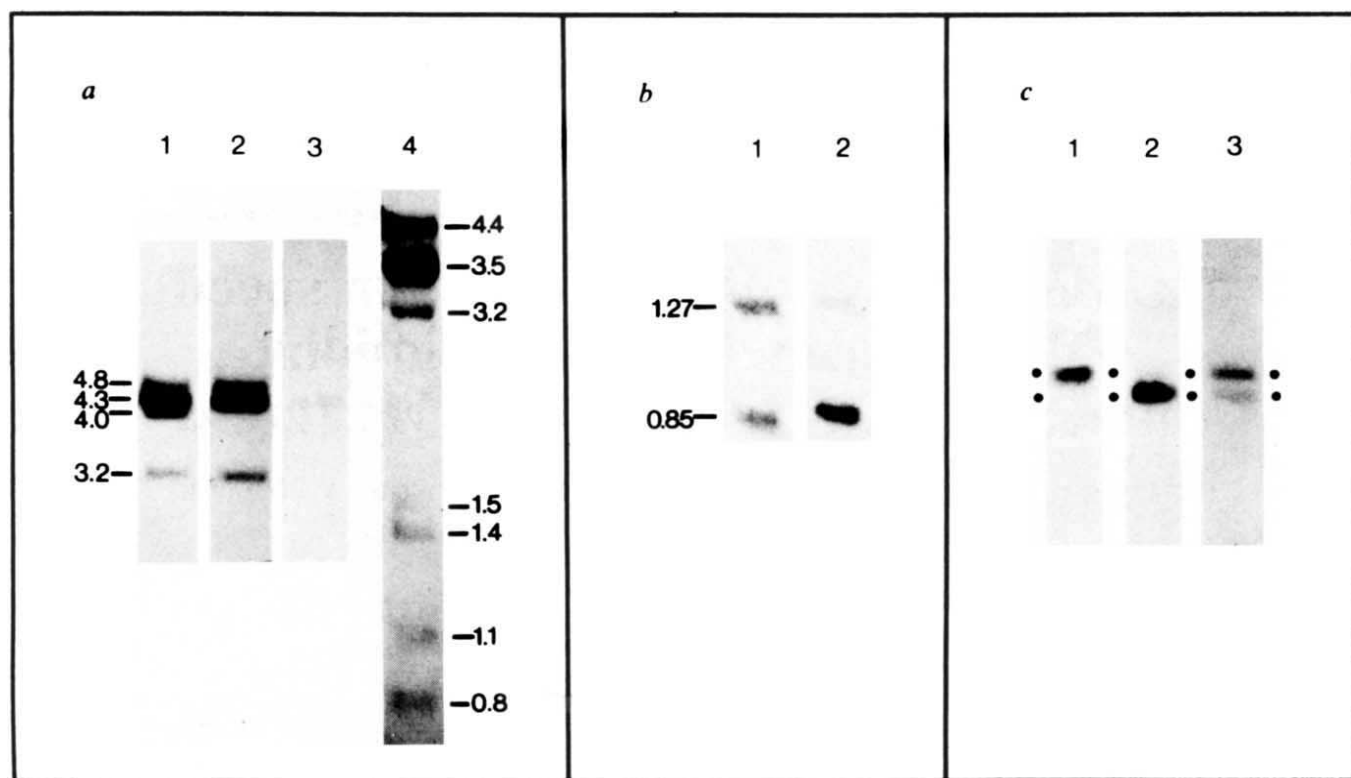


Fig. 5 Relative abundance of RNA transcribed from various regions of the ovalbumin clones. Each experiment follows a similar protocol. After digestion with restriction enzymes, 1–2 µg of DNA was electrophoresed through an agarose gel and transferred to nitrocellulose²⁸ (a) or DBM-paper²⁹ (b and c). Filters were then hybridized to ³²P-labelled RNA prepared from DNA-injected oocytes as described for Table 1. Between 2 × 10⁵ and 10 × 10⁵ acid-insoluble d.p.m. were used in each hybridization. For each fragment, bound DNA was in excess over complementary ³²P-labelled RNA. After hybridization for 1.5 to 3 d, filters were washed extensively, treated with DNase-free RNase (25 µg per ml) for 1 h at 37 °C, and exposed to hypersensitized film⁴⁰. To quantify hybridized radioactivity, two techniques were used. Either bands were cut out of the filter and bound radioactivity was determined in a scintillation counter (a), or autoradiograms were scanned with a Joyce-Loebl microdensitometer (a, c). Each hybridization was performed in duplicate. a. Transcripts from pOv12 (tracks 1 and 4), pOv8 (track 2), or pOv12 plus α-amanitin (1 µg per ml, track 3) were hybridized to the *Hind*III fragments of pOv12 (tracks 1, 2 and 3) or the *Hind*III-*Pst*I fragments of pOv12 (track 4). b. Transcripts from pOv230 and pOv12. ³²P-labelled RNA prepared from oocytes injected with pOv230 (track 1) or pOv12 (track 2) was hybridized to the *Hph*I fragments of pOv230. Only the relevant region of the fel is shown. The amount of ³²P-RNA hybridized per kilobase of DNA was determined by microdensitometry. The values obtained for the 0.85-kilobase fragment were set to 100% in both hybridizations. Relative hybridization to the 1.27-kilobase fragment was: track 1, 85%; track 2, 18%. Thus the level of stable RNA derived from the 1.27-kilobase *Hph*I pOv230 fragment is 4.7-fold higher in pOv230-injected oocytes. c. Transcription of both the sense and non-sense strands of pOv230. Separated strands of the 1.27-kilobase *Hph*I pOv230 fragment were prepared by electrophoresis of the purified, alkali-denatured fragment through a cold 1.4% agarose gel as described⁴². The two strands (present in equal amounts in the same gel) were then transferred to DBM-paper²⁹. Black dots indicate the position of the two strands on the filter. Filters were hybridized to the following radioactive probes. Track 1: ³²P-labelled ovalbumin mRNA (prepared by terminal labelling of mRNA fragments generated by boiling for 15 min in formamide), track 2: single-stranded ovalbumin cDNA synthesized *in vitro* from ovalbumin mRNA using a 20-min reverse transcriptase reaction in the presence of 4 mM sodium pyrophosphate; track 3: ³²P-labelled RNA prepared from oocytes injected with pOv230.

mRNA would be present at (0.5)⁶, or 1.6% of the VP1 mRNA level. A second explanation is that the transcription initiation and termination signals in the late region of SV40 might be more efficiently recognized by *Xenopus* oocytes than are those in the ovalbumin gene, resulting in greater translational efficiency of SV40 transcripts.

Most pOv12 transcripts do not both initiate at the cap site and terminate at the polyadenylation site. Rather, we suspect for the following reasons that most are produced either by random transcription initiation and termination throughout the template, or by transcription initiated within the vector which reads through into the inserted ovalbumin gene. (1) The level of transcripts from the vector is higher than the level of transcripts from the ovalbumin gene in the same plasmid; (2) transcripts from all regions of the inserted chicken DNA, including those lying outside the ovalbumin gene, are present in similar amounts; (3) no more stable RNA is synthesized

from pOv12 than from pBR322, and (4) electron microscopy of pBR322 transcription complexes by the Miller technique often reveals transcripts much longer than pBR322 itself (M. Trendelenburg and J.B.G., unpublished results). Although these data are all consistent with readthrough transcription, none provides compelling proof of it, and one observation could be taken to argue against it: the ovalbumin gene is inserted in opposite orientations in pOv8 and pOv12, yet the two templates direct ovalbumin synthesis with comparable efficiencies. If transcription of the chromosomal gene is due solely to readthrough transcription from the vector, then it must occur in both directions.

We thank Susan Whytock and Philippa Black for assistance, and R. A. Laskey, E. M. de Robertis, L. J. Korn, J. R. E. Wells and D. A. Melton for advice. M. P. W. is a Fellow of the Helen Hay Whitney Foundation.

Received 17 December 1979; accepted 17 April 1980.

1. Mertz, J. E. & Gurdon, J. B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1502-1506 (1977).
2. Melton, D. A. & Cortese, R. *Cell* **18**, 1165-1172 (1979).
3. De Robertis, E. M. & Olson, M. V. *Nature* **278**, 137-143 (1979).
4. De Robertis, E. M. & Mertz, J. E. *Cell* **12**, 175-182 (1977).
5. Rungger, D. & Turler, H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6073-6077 (1978).
6. Probst, E., Kressmann, A. & Birnstiel, M. L. *J. molec. Biol.* **135**, 709-732 (1979).
7. Crawford, L. V. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 117-121 (1978).
8. Reddy, V. B. *et al. Science* **200**, 494-502 (1978).
9. Fiers, W. *et al. Nature* **273**, 113-120 (1978).
10. Dugaiczky, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2253-2257 (1979).
11. Gannon, F. *et al. Nature* **278**, 428-434 (1979).
12. McReynolds, L., Catterall, J. & O'Malley, B. W. *Gene* **2**, 217-231 (1977).
13. Lai, E. C., Woo, S. L. C., Bordon-Riser, M. E., Fraser, T. H. & O. Malley, B. W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 244-248 (1980).
14. Lai, E. *et al. Cell* **18**, 829-842 (1979).
15. Roop, D. R., Tsai, M. J. & O'Malley, B. W. *Cell* **19**, 63-68 (1980).
16. O'Farrell, P. J. *biol. Chem.* **250**, 4007-4021 (1975).
17. Lee, Y. & Montgomery, R. *Archs Biochem. Biophys.* **97**, 9-17 (1962).
18. Milstein, C. *Biochem. J.* **110**, 127-134 (1968).
19. Lane, C. D. & Knowland, J. S. In *The Biochemistry of Animal Development IV* (ed. Weber, R. A.) (Academic, New York, 1975).
20. Lane, C., Shannon, S. & Craig, R. *Eur. J. Biochem.* **101**, 485-495 (1979).
21. Deacon, N. & Ebringer, A. *FEBS Lett.* **79**, 191-194 (1977).
22. Gurdon, J. B. & Brown, D. D. *Devl Biol.* **67**, 346-356 (1978).
23. Lai, C. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 71-75 (1979).
24. Gruss, P., Lai, C., Dhar, R. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4317-4321 (1979).
25. Hamer, D., Smith, K., Boyer, S. & Leder, P. *Cell* **17**, 725-735 (1979).
26. Hamer, D. H. & Leder, P. *Cell* **18**, 1299-1302 (1979).
27. Stark, G. R. & Williams, J. G. *Nucleic Acids Res.* **6**, 195-203 (1979).
28. Southern, E. M. *J. molec. Biol.* **98**, 503-518 (1975).
29. Wahl, G. M., Stern, M., & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683-3687 (1979).
30. McReynolds, L. *et al. Nature* **273**, 723-728 (1978).
31. Miller, O. & Bakken, A. in *5th Karolinska Symp.*, 155-157 (Karolinska Institutet, Stockholm, 1972).
32. Trendelenburg, M. & Gurdon, J. B. *Nature* **276**, 292-294 (1978).
33. Buell, G., Wickens, M., Payvar, F. & Schimke, R. *J. biol. Chem.* **253**, 2471-2482 (1978).
34. Bailey, J. & Davidson, N. *Analyt. Biochem.* **70**, 75-85 (1976).
35. Gurdon, J. B., Lane, C. D., Woodland, H. R. & Marbaix, G. *Nature* **233**, 177-182 (1971).
36. Gurdon, J. B. *J. Embryol. exp. Morph.* **36**, 523-540 (1976).
37. Laskey, R. A., Mills, A. D., Gurdon, J. B. & Partington, G. A. *Cell* **11**, 345-351 (1977).
38. Rhoads, R., McKnight, G., & Schimke, R. *J. biol. Chem.* **248**, 2031-2039 (1973).
39. Laemmli, U. *Nature* **227**, 680-685 (1970).
40. Laskey, R. & Mills, A. D. *Eur. J. Biochem.* **56**, 335-341 (1975).
41. Hirs, C. *Meth. Enzym.* **11**, 325 (1967).
42. Perlman, D. & Huberman, J. A. *Analyt. Biochem.* **83**, 666-677 (1977).
43. Sutcliffe, J. G. *Cold Spring Harbor Symp. quant. Biol.* **43**, 77-90 (1978).

Cleavage of pyrimidine dimers in specific DNA sequences by a pyrimidine dimer DNA-glycosylase of *M. luteus*

William A. Haseltine, Lynn K. Gordon & Christina P. Lindan

Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts 21205

Robert H. Grafstrom, Nancy L. Shaper & Lawrence Grossman

Biochemistry Department, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

Pyrimidine dimer formation in response to UV radiation is governed by the thymine content of the potential dimer and the two flanking nucleotides. An enzymatic activity can be purified from Micrococcus luteus that cleaves the N-glycosyl bond between the 5' pyrimidine of a dimer and the corresponding sugar without rupture of a phosphodiester bond. We propose that strand scission at a dimer site by the M. luteus enzyme requires two activities, a pyrimidine dimer DNA-glycosylase and an apyrimidinic/apurinic endonuclease.

ULTRAVIOLET light creates potentially lethal and mutagenic damage in the DNA of exposed organisms. The principal photoproduct is the pyrimidine cyclobutane dimer formed between adjacent pyrimidines, producing thymine-thymine (T <=> T), thymine-cytosine (T <=> C), and cytosine-cytosine (C <=> C) dimers. Cells can recover from damage induced by UV light through various mechanisms including excision repair, enzymatic photoreversal, and post-replication repair^{1,2}. One process of excision repair proceeds via endonucleolytic strand scission in the vicinity of the photoproducts with subsequent

excision of dimers^{1,3}. Endonucleases that specifically cleave DNA near the site of pyrimidine dimers have been identified in several bacterial species⁴⁻⁷ in slime moulds⁸, rat liver⁹, and in mammalian tissue¹⁰. In most organisms repair of UV damage is multigenic².

We have investigated the effect of local sequence on the distribution of UV light induced photodamage in DNA. The effect of neighbouring sequences in the formation of such damage is important to an understanding of the mutagenic effect of UV radiation. Since previous studies have indicated

that local sequence may affect the formation of pyrimidine dimers¹²⁻¹⁵, it seemed likely that DNA sequencing methods could be used to measure the effect of neighbouring sequence on the distribution of photodimers. Accordingly, a UV endonuclease prepared from *Micrococcus luteus* was used to cleave DNA substrates of defined sequence that had been irradiated with UV light, and the products were analysed on high resolution polyacrylamide gels.

During these experiments we observed that the dimer-specific *M. luteus* endonuclease activity did not cleave the DNA 5' to the site of the dimer as originally suggested by other methods. Rather, we found that strand scission occurs at the site of a pyrimidine dimer by the combined action of two enzymatic activities, a pyrimidine dimer DNA-glycosylase that cleaves the glycosylic bond between the 5' pyrimidine of the dimer and its sugar, and an apyrimidinic/apurinic (AP) endonuclease.

Distribution of dimer damage within a defined sequence

DNA fragments of defined sequence were used as substrates for UV radiation and endonuclease cleavage. The DNA restriction fragments of defined sequence were obtained from a plasmid, pLJ3 (refs 16, 17). The DNA substrates were terminally labelled with ³²P at either the 5' or 3' end. Labelled DNA was first irradiated and then cleaved with the dimer-dependent endonuclease, partially purified as the Sephadex G-75 fraction from *M. luteus*⁷. Fragments resulting from endonucleolytic cleavage were layered onto denaturing, high-resolution polyacrylamide gels of the type used for DNA sequencing. To determine the length of the reaction products and, therefore, the precise site of enzymatic strand scission, the electrophoretic mobility of the cleavage products was compared to the migration rate of fragments produced by chemically sequencing the same strand¹⁸.

In an initial experiment, a DNA fragment labelled at the 5' terminus was irradiated with increasing doses (from 500 to 10,000 J m⁻² of 254-nm light (UV-DNA) from a germicidal lamp. High doses of UV radiation were used because the target size was small. The irradiated DNA was treated with concentrations of the endonuclease that had previously been shown to be saturating for the cleavage reactions. The products of these reactions were analysed by polyacrylamide gel electrophoresis and the resulting migration patterns were analysed by autoradiography (Fig. 1). Inspection of the gel reveals that the UV-DNA was cleaved by the endonuclease at specific sites and only in the vicinity of possible pyrimidine dimers. The endonuclease did not cleave unirradiated DNA (Fig. 1, lanes 1 and 2), and breaks were not apparent in the DNA irradiated with 10,000 J m⁻² and not exposed to enzyme (Fig. 1, lane 13). With increasing doses of UV radiation up to 3,500 J m⁻², the yield of cleavage products also increased.

In order to determine if the extent of dimer formation is sequence specific, similar experiments were performed using doses between 75 and 7,500 J m⁻². Quantitative data were obtained from experiments in which the DNA was irradiated, treated with the Sephadex G-75 fraction and layered onto denaturing gels. The amount of each product was determined by measuring the amount of radioactivity in each band. This calculation assumed that the enzyme cleaved quantitatively at all dimers. This assumption is justified, since previous experiments demonstrated that the dimer-dependent endonuclease of *M. luteus* cleaved DNA in response to all possible dimers, T $\diamond$ T, C $\diamond$ T, and C $\diamond$ C; also, the combined action of the UV endonuclease preparation and added exonucleases resulted in the complete removal of all dimers from the DNA^{3,19}. Furthermore, in the reaction conditions utilized in these experiments, increased amounts of enzyme did not lead to an increased number of breaks. The incision frequencies at each dimer do not reflect a property of the *M. luteus* enzyme alone, since the same distribution of

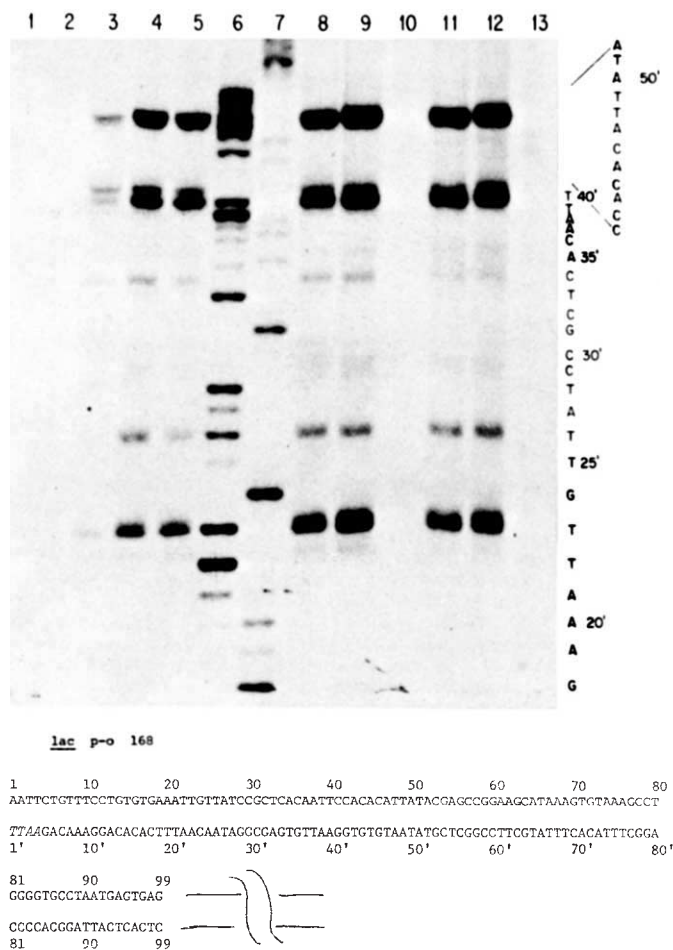


Fig. 1 Site-specific cleavage by the dimer dependent endonuclease on DNA irradiated with various doses of UV light. 168 base pair ³²P-5' end-labelled *lac p o* DNA^{16,17} was irradiated with the indicated amounts of 254-nm light and incubated with saturating amounts of the Sephadex G-75 fraction of the *M. luteus* enzyme. (Sephadex G-75 fraction: dimer-specific endonuclease containing both the pyrimidine dimer DNA-glycosylase and the AP endonuclease.) Samples were layered onto a urea-containing polyacrylamide gel. The DNA was heated for 2 min in 0.01 M Na₂HPO₄ pH 7.0 and 10% glycerol before loading the sample. The amount of endonuclease necessary to carry the reaction to completion was determined in separate experiments in which the extent of cleavage of the DNA fragment irradiated at 500 J m⁻² was determined as a function of enzyme concentration. These experiments showed that an increase in the concentration of the enzyme above 1 unit per 50 µl of reaction mix did not cause further scission. The reaction buffer contained 0.01 M Tris-HCl pH 7.4, 0.05 M NaCl, 0.001 M Na₂EDTA. Incubation was for 60 min at 37 °C. Reaction mixtures contained: unirradiated DNA treated with 3 units (lane 1) and 9 units (lane 2) of the Sephadex G-75 enzyme; DNA irradiated with 120 J m⁻² (lane 3), 480 J m⁻² (lane 4), 960 J m⁻² (lane 5), 1,440 J m⁻² (lane 8), 4,320 J m⁻² (lane 9), lane 10 is blank, 7,200 J m⁻² (lane 11), 10,000 J m⁻² (lane 12). Three units of enzyme were added to these reactions. DNA of lane 13 was exposed to 10,000 J m⁻² but no enzyme was added to the reaction. Lane 6 contains products of neocarzinostatin sequencing reactions producing cleavage at T's and A's²¹. Lane 7 contains products of dimethyl sulphate modification with cleavage at G's and A's¹⁸. Only the central portion of the gel is pictured here. The DNA sequence of the substrate is given above for the first 99 nucleotides. For this experiment the DNA was labelled at the 5' terminus in reactions that contained [γ -³²P]ATP and polynucleotide kinase in an exchange reaction³². In subsequent experiments the substrate was also labelled at the 3' terminus using [α -³²P]dATP and [α -³²P]dTTP in reactions that contained the Klenow fragment of *E. coli* DNA polymerase I³³. The unprimed numbers indicate the DNA strand labelled at the 5' terminus; the primed numbers DNA labelled at the 3' terminus. The italicized letters indicate the positions of the 3' labelled nucleotides.

enzyme sites was obtained in similar experiments using the dimer-dependent endonuclease V (ref. 6) purified from T4 infected *Escherichia coli* (data not shown).

The per cent incision at potential dimer sites as a function of the irradiation dose is plotted in Fig. 2a for representative sequences. Several conclusions can be drawn from this data.

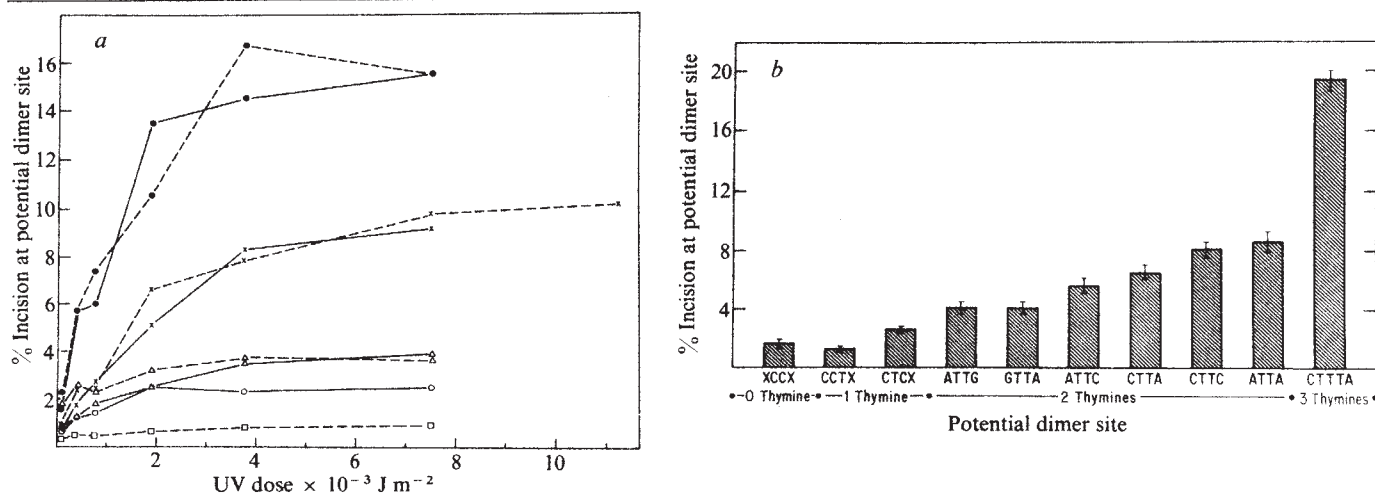


Fig. 2 Frequency of scission at potential dimer sites as a function of UV dose and sequence. *a*, Dose-response: *lac p-o* 117 (see below) and *lac p-o* 168 DNA (see Fig. 1) were used for this experiment. The DNA was irradiated with 254-nm light from a germicidal lamp at the indicated dose at a dose rate of $7.5 \text{ J m}^{-2} \text{ s}^{-1}$. The *M. luteus* endonuclease (Sephadex G-75 fraction)⁷ was used at a saturating dose such that the addition of more enzyme did not produce additional breaks. Three units of the enzyme were used in a 50- μl reaction mixture that contained 0.01 M Tris-HCl pH 7.4, 0.05 M NaCl, 0.001 M Na_2EDTA and the reactions were incubated at 37°C for 60 min. The DNA was precipitated and the fragments separated on 8%, 12% or 20% polyacrylamide gels that included 7 M urea; products of the same DNA fragments subject to the DNA sequencing reactions described by Maxam and Gilbert¹⁸ or treated with neocarzinostatin²¹ were layered on adjacent lanes of the gel to permit determination of the length of each scission product. The labelled DNA products were visualized by autoradiography. Because of the possibility that a single DNA molecule might contain more than one dimer, the values for per cent incision were determined using a correction factor. This empirical factor accounts for products that were removed from a band that corresponded to breakage at dimer site X by breakage of the molecules between site X and the labelled terminus. Thus,

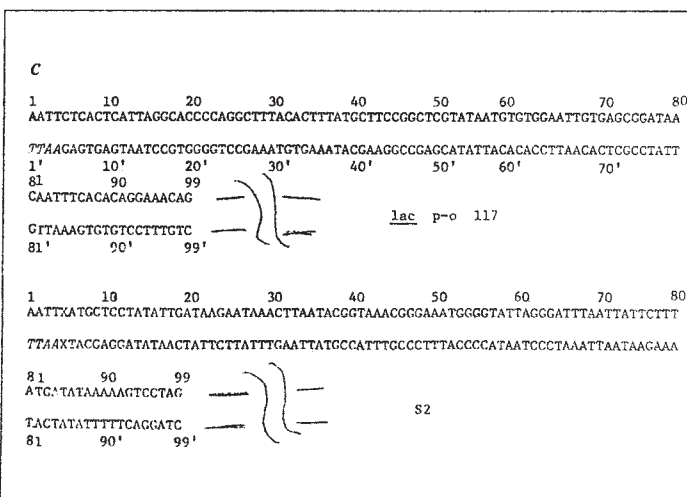
$$\text{per cent incision} = 100 \left[\frac{\text{c.p.m. site X}}{\text{c.p.m. total}} + \frac{\text{c.p.m. faster than site X}}{\text{c.p.m. total}} \times \frac{\text{c.p.m. site X}}{\text{c.p.m. total}} \right]$$

The c.p.m. total was determined by Cherenkov counting of the total material in each lane of the gel. The time of the electrophoresis was such that all the radioactive material of each lane was present on the gel. The location within the DNA substrates of the potential dimers that were used to construct this figure are indicated below. The values are those deduced from the amount of radioactivity in the band that corresponds to a scission event at the central dimer only, except in the case of the sequence CTTTA. This value is a summation of the total incision frequency at the two possible central dimers. The relative rate of incision was approximately equal at each of the two possible thymine dimers of this sequence.

●	CTTTA:	<i>lac p-o</i>	168	3'	73'-77'
●	CTTTA:	<i>lac p-o</i>	168	3'	66'-70'
×	ATTA:	<i>lac p-o</i>	117	3'	54'-57'
×	ATTA:	<i>lac p-o</i>	117	5'	12-15
△	ATTG:	<i>lac p-o</i>	117	3'	80'-83'
△	ATTG:	<i>lac p-o</i>	117	5'	65-68
○	TTCA:	<i>lac p-o</i>	168	3'	17'-20'
□	CCCC:	<i>lac p-o</i>	117	5'	20-24

b, Sequence dependence: Three DNA fragments labelled at either the 5' or 3' end were used for this experiment. The DNA was irradiated with 254 nm light from a germicidal lamp at $7,500 \text{ J m}^{-2}$ at a dose rate of $7.5 \text{ J m}^{-2} \text{ s}^{-1}$. UV-irradiated DNA was cleaved with the endonuclease (Sephadex G-75 fraction) and analysed as in *a*. The error bars indicate $\pm$ s.e.m. as calculated from the number of independent measurements at each site as indicated in parentheses. The following numbers indicate the location of the dimer within the sequences. Sequences are listed in the 5' to 3' direction.

XCCX (13):	117: 20-23 (7)	ATTG (12):	117: 63'-66' (4)
	168: 28-31 (2)		168: 38-41 (5)
	168: 56-59 (2)		S-2: 73-76 (1)
	S-2: 53'-56' (2)		S-2: 24'-27' (2)
CCTX (8):	117: 14'-17' (2)	CTTA (9):	S-2: 31-34 (7)
	117: 23'-26' (2)		S-2: 21'-24' (2)
	168: 78-81 (2)		
	168: 87-90 (2)		
CTCX (12):	117: 9-12 (2)	CTTC (10):	117: 41-44 (9)
	117: 48-51 (2)		168: 60'-63' (1)
	117: 69'-72' (2)		
	168: 32-35 (3)		
	168: 53'-56' (3)		
ATTG (24):	117: 65-68 (8)	ATTA (20):	117: 12-15 (4)
	117: 80'-83' (2)		117: 54'-57' (4)
	168: 21-24 (6)		168: 47-50 (6)
	168: 36'-39' (3)		S-2: 58-61 (2)
	S-2: 16-19 (5)		S-2: 70-73 (2)
			S-2: 33'-36' (2)
GTTA (8):	117: 77'-80' (2)	CTTTA (31):	117: 27-31 (14)
	168: 24-27 (6)		117: 34-38 (11)
			168: 66'-70' (3)
			168: 73'-77' (3)



Up to six sites were used to calculate each value in panel *b* and the value of each sequence was determined in at least eight independent measurements. At some sequences the values were obtained using DNA that had been labelled at either the 5' or the 3' terminus, and in either case the calculated incision frequency was the same. The s.e.m. was within $\pm 10\%$ of the values indicated for each sequence regardless of its location within the DNA fragments. The sequences used for this work were the *lac p-o* 168 DNA fragment (see legend to Fig. 1) the *lac p-o* 117 fragment^{16,17} and DNA fragment designated S2. The sequence of these substrates is indicated in panel *c*. S2 is derived from the pLJ3 plasmid^{16,17} by terminal labelling of the *EcoRI* digested plasmid followed by cleavage with *HaeIII*. S2 is a DNA fragment approximately 350 nucleotides long. The sequence shown was determined by B. Royer-Pokora and R. Martin (unpublished observations).

For some potential dimer sites, the extent of dimer formation increased with increasing doses between 75 and $3,500 \text{ J m}^{-2}$. At doses greater than $3,500 \text{ J m}^{-2}$, a plateau level for the extent of dimer formation was reached at every potential dimer site. The per cent incision observed at the plateau level probably reflects the photo-steady state level that is governed by the relative rates of dimer formation and photoreversal.

Figure 2a also demonstrates that there are measurable differences in the extent of dimer formation at different sites. To investigate the effect of neighbouring sequences, the extent of dimer formation was determined for 38 different possible dimer sites at a dose of $7,500 \text{ J m}^{-2}$, at which a photo-steady state was reached. The results are presented in Fig. 2b.

The primary determinant of the extent of dimer formation at adjacent pyrimidines is the thymine content of the sequence. Less than 3% of the sequences that contain cytosines form dimers even at the highest doses of radiation. This low level may reflect both the slow forward rate of cytosine-cytosine dimer formation and the rapid reversal rate or photolysis of these dimers²⁰. It is noteworthy that in these conditions the photosteady state level was reached when only a small fraction of the pyrimidines were dimerized. For example, thymine-cytosine dimers reached a steady state when 1 to 3% of the potential sites were dimerized. The actual value for dimer production is affected by the two nucleotides that flank the dimer with the extent of dimerization varying from 4% at the sequence ATTG to almost 9% at the sequence ATTA. There is no major effect of sequence composition other than that produced by the nucleotides that immediately flank the dimer.

For example, the extent of dimer formation in the underlined dimers is the same for the sequence TGGAATTGTGAG (*lac p-o* 117, 61–72) and CCTATATTGATTAA (S2, 11–24). Figure 2a also shows that the dose-response curves as well as the plateau levels are similar for identical sequences, regardless of their location within the DNA substrate.

The site of enzymatic cleavage

The electrophoretic mobilities of products resulting from endonuclease cleavage of UV-DNA were unexpected. Previous biochemical experiments indicated that the site of endonucleolytic breakage of UV-DNA places the photoproduct at the 5' end of the phosphodiester bond break^{7,19}. According to Fig. 1, however, scission of the DNA at a pyrimidine dimer results in DNA fragments that had electrophoretic mobilities similar to those of DNA sequencing reactions in which a 3' pyrimidine would have been eliminated. The sequencing reactions with 5' end-labelled DNA fragments result in species that are phosphorylated at the 3' terminus^{18,21}. Therefore, comparable mobility of the enzyme-treated DNA might imply that the endonuclease had cleaved the UV-DNA between the two thymines of a dimer. However, the cyclobutane bond of the dimer remains intact after incision of the DNA by the *M. luteus* enzyme²². It seemed unlikely that the enzymatic and chemical sequencing products were identical, even though they displayed similar electrophoretic mobilities.

One explanation for the anomalous mobility of the enzymatic cleavage products was that the 3' termini of these

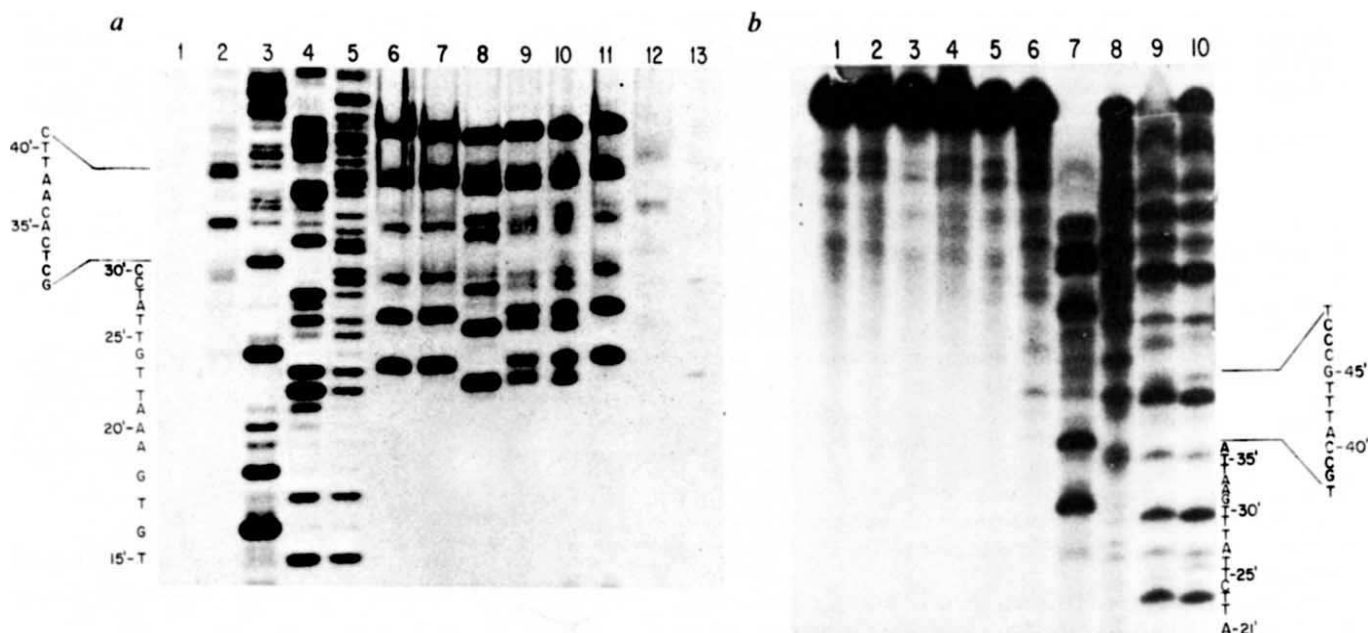


Fig. 3 Effect of alkaline hydrolysis on the electrophoretic mobility of 5' and 3' end-labelled DNA scission products. *a*, 5' End-labelled *lac p-o* 168 DNA was used for these experiments. Where indicated, DNA was irradiated with 480 J m^{-2} (UV-DNA). Lane 1, unirradiated DNA was heated in 0.1 M NaOH at 90°C for 30 min (heat/alkali treatment) and layered on the gel in a buffer that contained 0.05 M NaOH 7 M urea. Lane 2, UV-DNA treated as in lane 1. Lanes 3, 4 and 5, unirradiated DNA treated with either dimethyl sulphate (DMS)¹⁸ to generate cleavage at positions of G's and to a lesser extent at A's (lane 3), neocarzinostatin to produce cleavage at T's and to a lesser extent at positions of A's²¹ (lane 4), and hydrazine¹⁸ to cleave at positions of C's and T's (lane 5). Lanes 6–11 contain UV-DNA treated with 3 units of the Sephadex G-75 fraction of the *M. luteus* endonuclease as described in Fig. 1. The loading buffer was 0.01 M Na_2HPO_4 pH 7.0, 10% glycerol (lane 6) or 0.05 M NaOH, 7 M urea (lane 7). The DNA in lane 8 was heated in 0.1 M NaOH at 90°C for 30 min before loading in a solution of 0.1 M NaOH, 10% glycerol. Lane 9 contains DNA products which were heated at 37°C for 30 min in 0.1 M NaOH before loading in a solution of 0.1 M NaOH, 10% glycerol. Lane 10 contains DNA products heated at 90°C for 30 min in 0.1 M Na_2HPO_4 pH 7.0 and loaded in that buffer plus 10% glycerol. Lane 11 contains the reaction products heated at 37°C for 30 min in 0.01 M Na_2HPO_4 pH 7.0 and layered directly in a buffer that contained 0.01 M Na_2HPO_4 , 10% glycerol. (Note that this lane demonstrates that cleavage of the phosphodiester bonds occurs in DNA treated with the Sephadex G-75 enzyme fractions alone. Heat or alkaline treatment of the product are not required for DNA breakage with this preparation.) Lane 12, UV-DNA treated in 0.01 M Na_2HPO_4 pH 7.0 at 90°C for 30 min and loaded in 0.01 M Na_2HPO_4 , 10% glycerol. Lane 13, unirradiated DNA incubated with the Sephadex G-75 fraction and loaded in a solution that contained 0.05 M NaOH, 7 M urea. Note that the 5' to 3' orientation of the DNA fragments on polyacrylamide gels of *a* and *b* are different due to labelling the fragments at the 5' or 3' ends. *b*, 3' End-labelled S2 DNA was used for this experiment. Unirradiated DNA was layered onto the gel without (lane 1) and with (lane 2) treatment at 90°C for 30 min in 0.1 M NaOH (heat/alkali). Unirradiated DNA treated with 3 units of the Sephadex G-75 fraction of the *M. luteus* enzyme was also layered on the gel before (lane 3) and after (lane 4) the heat/alkali treatment. DNA irradiated with $7,500 \text{ J m}^{-2}$ of UV light was layered onto the gel before (lane 5) and after (lane 6) heat/alkali treatment. UV-irradiated DNA ($7,500 \text{ J m}^{-2}$) was treated with 3 units of the enzyme and layered directly onto the gel (lane 9) or after heat/alkali treatment (lane 10). Unirradiated DNA was treated with DMS (lane 7) or with neocarzinostatin (lane 8). All samples were loaded in 0.05 M Tris-HCl pH 7.5, 0.05 M boric acid, 0.001 M Na_2EDTA pH 8.3 and 7 M urea.

Table 1 Sodium borohydride reduction of apyrimidinic site produced by pyrimidine dimer DNA-glycosylase

Sample		Per cent breakage after treatment	
		- Alkaline hydrolysis	+ Alkaline hydrolysis
Native DNA + PyDNG	Control	15	18
	NaBH ₄ Reduced	15	15
Apyrimic DNA	Control	25	90
	NaBH ₄ Reduced	17	16
UV DNA	Control	18	22
	NaBH ₄ Reduced	16	17
UV DNA + PyDNG	Control	29	86
	NaBH ₄ Reduced	23	24

³H-Labelled ΦX174 RFI DNA was either untreated, UV-irradiated at 30 J m⁻² or made apurinic as described in the legend to Fig. 4. Assays contained 4.0 nmol of the appropriate DNA, and 14 units of the pyrimidine dimer DNA-glycosylase (PyDNG, the CM-cellulose fraction) in 400 μl of buffer A. Incubations were performed at 37 °C for 20 min and stopped by heating at 70 °C for 15 min. The pH was adjusted to 6.5 with 1 M acetic acid. 2 M K₂HPO₄ (pH 6.5) was added to 0.5 M. Samples were split into two equal portions and one was reduced with 0.3 M NaBH₄. Samples were then assayed for alkaline sensitive sites by treating with 2 ml of alkaline denaturation buffer for 5 min at room temperature or for 90 min at 37 °C. They were then neutralized with 0.4 ml of 2.0 M Tris-HCl (pH 4.0) and the conversion of RFI to RFII measured as described in Fig. 4. The numbers indicate the per cent of the substrate molecules that sustained at least one single-stranded break after the indicated treatments.

DNA fragments were neither phosphoryl groups, such as those produced in the DNA sequencing reactions, nor hydroxyl groups. An apyrimidinic deoxyribose at the 3' terminus could result in the observed electrophoretic mobility of the cleavage product. To test the possibility that the cleavage product had a 3'-apyrimidinic terminus, a group sensitive to β-elimination conditions, UV-DNA was first incubated with the enzyme preparation and subsequently treated in 0.1 M NaOH at 90 °C for 30 min. The DNA was then analysed on polyacrylamide gels as before. Figure 3a demonstrates that the heat/alkali treatment alters the electrophoretic mobility of the cleavage products so that they migrate as if they were one nucleotide shorter (compare Fig. 3a, lanes 7 and 8). Identical results were also obtained when enzyme-treated DNA was heated with 0.5 M piperidine at 90 °C for 30 min (another agent that promotes β-elimination, data not shown). A mixture of both slow- and fast-migrating fragments was observed at each isolated thymine dimer sequence if less stringent post-enzyme hydrolysis conditions were used (37 °C in 0.1 M NaOH for 30 min) before suspension in loading buffer (Fig. 3a, lane 9). Both fragments were also obtained on heating of enzyme-treated DNA at neutral pH (Fig. 3a, lane 10), whereas incubation at 37 °C at neutral pH produced no shift in migration (Fig. 3a, lane 11). These experiments suggested that the 3' terminus might be composed of an apyrimidinic site which would have been associated originally with the 5' thymine moiety of the dimer.

Similar experiments were performed using DNA fragments labelled at the 3' terminus. The electrophoretic mobility of these cleavage products was unaffected by the heat and alkaline hydrolysis conditions, which resulted in altered mobilities of the 5' end-labelled DNA products (Fig. 3b). These experiments demonstrate that the 5' terminus of the break does not have a structure that is labile to conditions that promote β-elimination. Furthermore, these experiments also show that the increased electrophoretic mobility of the 5' end-labelled DNA upon alkaline hydrolysis is not an artefact of either UV radiation of DNA or of general β-elimination conditions.

Figure 3a (lane 2) shows that heavily irradiated DNA sustains damage that results in strand scission only upon treatment of the DNA with 0.1 M NaOH at 90 °C. The length

of these cleavage products suggests that such breaks occur predominantly at positions of cytosine and to a lesser extent at adenosine. The nature of these lesions is currently under investigation.

Purification of a pyrimidine dimer DNA-glycosylase

It had been previously reported that the *M. luteus* endonuclease cleaves DNA such that the pyrimidine dimer is at the 5' terminus of a phosphodiester bond break generating 3'-hydroxyl and 5'-phosphoryl groups. Analysis of the electrophoretic mobilities before and after alkaline hydrolysis

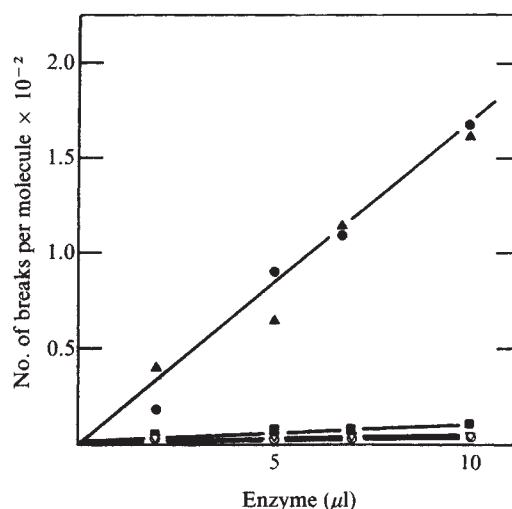


Fig. 4 Activity of pyrimidine dimer DNA-glycosylase for UV-irradiated DNA. The purification of the activity used in these experiments is given below. One unit is the amount of enzyme necessary to produce 1 break per nmol of UV-damaged ³H-labelled-ΦX174 RFI DNA for 20 min at 37 °C in 100 μl of buffer A which is 0.05 M Na-HEPES (pH 7.6), 0.001 M Na₂EDTA, 0.005 M MgCl₂ when coupled to alkaline hydrolysis or addition of an excess of human AP endonucleases. Assay conditions for these activities are described below.

	Protein (mg ml ⁻¹)	Activity (Units ml × 10 ⁻³)	
		DNA-glycosylase	AP endo
Phosphocellulose	8	360	—
Sephadex G-75	0.05	46.4	3.1
Hydroxylapatite	0.002	13.9	0.53
CM-cellulose	—	17.0	0.18

	Specific activity (Units μg ⁻¹)		Ratio of DNA-glycosylase to AP endonuclease
	DNA-glycosylase	AP endo	
Phosphocellulose	45	—	—
Sephadex G-75	928	62	15
Hydroxylapatite	6,950	265	26
CM-cellulose	—	—	94

ΦX174 RFI DNA was: untreated; heated at 70 °C for 15 min in 0.1 M NaCl, 0.01 M Na acetate (pH 5.0) to make apurinic DNA³⁴; or UV-irradiated at a dose of 30 J m⁻². Assays containing 1.0 nmol of the appropriate DNA in 100 μl of buffer A with the indicated volume of enzyme were incubated for 20 min at 37 °C and stopped by the addition of 2.0 ml of alkaline denaturation buffer (0.1 M Na₂HPO₄, 0.3 M NaCl, 0.025 M Na₂EDTA, pH 11.7). After 5 min at room temperature, the samples were neutralized in 0.4 ml of 2 M Tris-HCl (pH 4.0). In these conditions RFII and linear molecules are irreversibly denatured while RFI renatures. The conversion of RFI to RFII is then measured by filtering through nitrocellulose filters (Schleicher and Schuell BA85) which bind the denatured single strands of RFII DNA^{7,19}. The numbers of breaks per molecule were calculated from the Poisson distribution $N = -\ln z$, where z is the fraction of molecules with no breaks. △, Untreated DNA + DNA glycosylase + AP endonuclease; ○, apurinic DNA + DNA glycosylase; ■, UV-DNA + DNA glycosylase; ▲, UV-DNA + DNA glycosylase + AP endonuclease; ●, UV-DNA + DNA glycosylase + alkaline hydrolysis (37 °C, 90 min in alkaline denaturation buffer).

suggested a different mechanism of enzymatic cleavage. The electrophoretic gel patterns of the cleavage products could be produced by cleavage of the *N*-glycosylic bond between the 5' pyrimidine moiety of the dimer and the corresponding deoxyribose, as well as cleavage of the phosphodiester bond 3' to the apyrimidinic sugar moiety. The dimer would still be located at the extreme 5' end of the broken chain in this suggested mechanism. Therefore, our data implied that two different enzymatic activities were involved in the strand scission event, a DNA-glycosylase and an apurinic/apyrimidinic (AP) endonuclease.

The possibility that the Sephadex G-75 fraction of the *M. luteus* enzyme used for these experiments contained an AP endonuclease activity was investigated using Φ X174 RFI (a double-stranded covalently closed circular DNA molecule) that contained apurinic sites as a substrate. The Sephadex G-75 fraction contained such an activity when judged by conversion of RFI to nicked circular (RFII) and linear forms (see legend to Fig. 4). The Sephadex G-75 fraction was further purified to resolve the AP endonuclease from the DNA-glycosylase activity. Since treatment of UV irradiated DNA with enzyme fractions lacking the endogenous AP endonuclease would not result in direct breakage of phosphodiester bonds, DNA treated with the purified enzyme fractions was assayed for strand scission of Φ X174 UV irradiated RFI DNA by including an excess of the homogeneous AP endonuclease from human placenta²³. Using this assay, an enzymatic activity was purified from the Sephadex G-75 fraction by sequential column chromatography on hydroxylapatite and CM-cellulose. The relative level of AP endonuclease activity was substantially diminished in the CM-cellulose fraction which contained as a major component the pyrimidine dimer DNA-glycosylase activity. (Py-DNG) (see legend to Fig. 4).

The CM-cellulose fraction exhibited an optimum activity for alkaline-dependent breakage of UV-irradiated DNA between pH 6.5 and 8.0 which was stimulated by the presence of 50 to 100 mM salt. The catalytic activity was seven times greater in the presence of $MgCl_2$ than in the presence of EDTA but was not stimulated by $CaCl_2$, $ZnCl_2$ or $MnCl_2$. The dimer DNA-glycosylase activity was unaffected by the presence of ATP in the range 0.2 to 5.0 mM and no detectable exonuclease or RNase activity could be demonstrated in this fraction. Previously, it had been reported^{7,19} that the Sephadex G-75 fraction could be separated into two distinct 'UV endonucleases' by chromatography on DNA-cellulose. It has since been found that, during elution of the protein from a DNA-cellulose column, the DNA-glycosylase activity was preceded by a Mg^{2+} -dependent AP endonuclease and was followed by an AP endonuclease that was active in the absence of magnesium and in the presence of EDTA. In examining gross endonucleolytic activities specific for pyrimidine dimers, this phenomenon resulted in what appeared to be two chromatographically separable and unique 'UV endonucleases'⁷.

To determine if the more highly purified activity recognized photodimers rather than other types of UV damage, DNA exposed to low doses of UV radiation was treated with the DNA-glycosylase in the presence of an excess of human AP endonuclease²³. The number of DNA breaks was found to be directly proportional to the dose of UV light and hence to the number of dimers present. Pretreatment of the UV-irradiated DNA with photolyase purified from yeast and with long wavelength UV light, a treatment that specifically reverses pyrimidine dimers²⁴, produced DNA which no longer acted as a substrate for the enzyme. DNA irradiated with long-wavelength UV light in the presence of acetophenone, a photosensitizer that produces predominantly thymine dimers^{25,26}, was able to act as a substrate for the enzyme.

To determine the amount of phosphodiester bond cleavage of UV-irradiated DNA in the presence and absence of excess

AP endonuclease activity, UV-irradiated Φ X174 RFI was incubated with increasing concentrations of the DNA glycosylase. Figure 4 shows that the conversion of RFI to RFII and linear forms was much less in the absence of added AP endonuclease. The low but detectable level of strand scission still observed may be attributed to the low level of AP endonuclease still present in this preparation (legend to Fig. 4). Treatment of UV-irradiated RFI with the DNA-glycosylase modified the DNA such that it was converted to RFII and linear forms on alkaline hydrolysis (Fig. 4).

The above experiments suggested that the enzyme created an intermediate that contained an AP site. To test this possibility further, UV-irradiated DNA incubated with the CM-cellulose fraction was treated with sodium borohydride. Such treatment should reduce the potential aldehyde of the deoxyribose to the corresponding alcohol and thus stabilize the AP DNA to mild alkaline hydrolysis as was observed (Table 1). Reduction with sodium borohydride also stabilized²³ the UV-irradiated DNA that had been treated with the CM-cellulose enzyme (Table 1). Therefore, we conclude that treatment of UV-irradiated DNA with the CM-cellulose fraction creates an apyrimidinic site in the absence of phosphodiester bond hydrolysis.

Sites of DNA strand scission produced by the more purified CM-cellulose fraction were compared to those produced by the Sephadex G-75 fraction. 5' End-labelled UV-irradiated DNA was incubated with either enzyme fraction and then treated with 0.1 M NaOH for 30 min at 90°C. In these

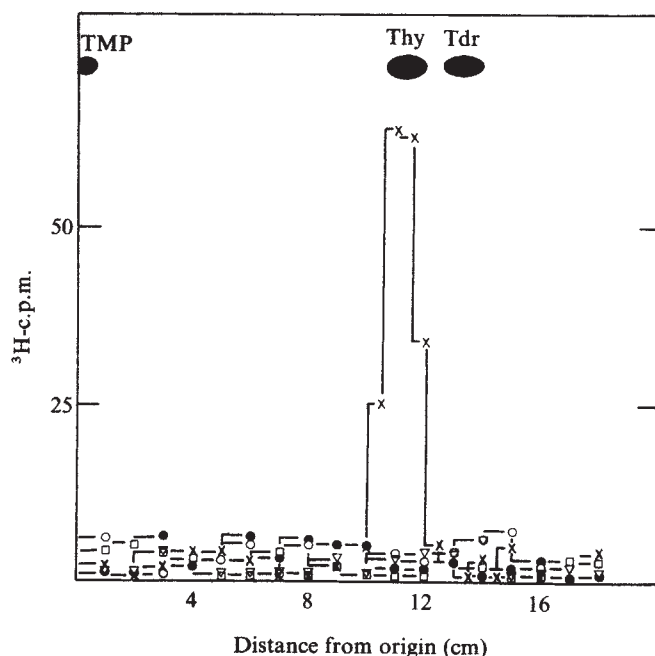


Fig. 5 Release of thymine by the pyrimidine dimer DNA-glycosylase and yeast photolyase. Assays containing 11 nmol of 3H -labelled Φ X174 RFI DNA (11,000 c.p.m. per nmole of nucleotide equivalent) that was either untreated or exposed to $600 J m^{-2}$ of UV irradiation were incubated at 37°C for 15 min in the presence of 400 units of the dimer DNA-glycosylase (hydroxylapatite fraction) in 500 μ l of buffer A (see Fig. 4). Samples were adjusted to 6 mM Na_2EDTA , 10 mM mercaptoethanol, and 150 mM NaCl. 800 Units of photolyase were added and samples were incubated at 30°C for 90 min in the presence of a black light. Samples were analysed for the release of 3H -thymine by diluting with 2 ml of H_2O and passing over a 0.5 ml QAE Sephadex (Cl) column equilibrated with H_2O , washed twice with 1 ml aliquots of 10 mM Na-HEPES, 2 mM Na_2EDTA buffer (pH 7.6). The flow through and washes were pooled and the samples were desalted over charcoal, analysed for thymine on PEI-cellulose plates in H_2O . This procedure separates thymine from thymidine, TMP, and thymine dimers. $\square$, UV-DNA + photolyase + black light; $\circ$, UV-DNA + DNA-glycosylase + black light; ∇ , UV-DNA + DNA-glycosylase + photolyase; $\times$, UV-DNA + DNA-glycosylase + photolyase + black light; $\bullet$, untreated DNA + DNA-glycosylase + photolyase + black light.

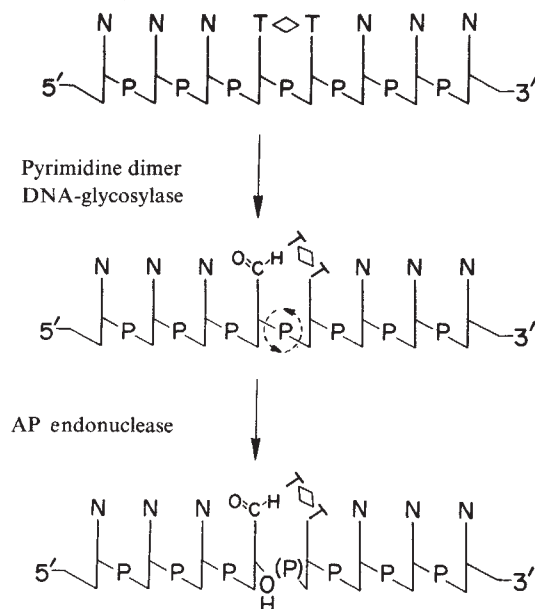


Fig. 6 A two-step model for incision at sites of pyrimidine dimers.

conditions, both enzyme preparations cleaved the irradiated DNA at the same sites and with the same relative frequency (data not shown).

The production of an AP site in UV-DNA treated with the dimer DNA-glycosylase proves that the enzyme cleaves an *N*-glycosylic bond. The electrophoretic mobility of the labelled cleavage products suggests that the bond broken is only that of the 5' pyrimidine moiety of the dimer. If this mechanism is correct, then the structure of the photoproduct should be that of a mixed thymine-thymidylate dimer that is still attached to the DNA. Photoreversal of such a structure should release free thymine. To test this possibility, UV-DNA that had been treated with the DNA-glycosylase was subsequently treated with yeast photolyase in the presence of visible light. Free thymine was released in the reactions that contained the yeast photolyase but only on exposure to visible light (Fig. 5). The amount of free thymine released by the enzymatic photolysis was approximately 70% of that expected if only one of the thymines of every dimer were released. Treatment of UV-DNA labelled with ^3H -thymine with the glycosylase alone did not release any labelled thymine. Had ^3H -thymine dimers been released, they would have remained at the origin in this chromatographic system. Similar results were obtained by UV photolysis with a second irradiation of the DNA-glycosylase treated DNA with 254-nm light (data not shown). Again, in these experiments thymine was only released from the UV-irradiated DNA by photoreversal after pre-treatment of the UV-irradiated DNA with the DNA-glycosylase. Moreover, thymine was not released from unirradiated DNA treated with the DNA-glycosylase followed by photoreversal. From these experiments we conclude that the CM-cellulose fraction contains an activity that cleaves the *N*-glycosylic bond between the 5' pyrimidine of the dimer and its sugar.

Discussion

The experiments presented here demonstrate that dimer formation is governed by (1) the number of adjacent thymines and (2) the nature of the nucleotides that flank the dimer. Thus the sequence of a potential target site can influence the frequency of mutation induced by UV light at the site.

The experiments also demonstrate that cleavage of the UV-irradiated DNA with the *M. luteus* enzyme occurs at the site of the dimer itself rather than 5' to the dimer as proposed

previously. As a result, it has been possible to purify a 'pyrimidine dimer DNA-glycosylase' activity from the *M. luteus* UV endonuclease preparation. This activity creates an AP site in the UV-irradiated DNA but does not rupture the phosphodiester backbone. After treatment of UV-DNA with the pyrimidine dimer DNA-glycosylase, free thymine can be released from UV-irradiated DNA upon photoreversal of the mixed thymine thymidylate dimer with either yeast photolyase or photolysis. Based on this evidence, we propose that the cleavage activity of UV-irradiated DNA observed in the less purified Sephadex G-75 fraction of the *M. luteus* endonuclease fraction occurs via the combined action of two enzymatic activities as shown in Fig. 6. The pyrimidine dimer DNA-glycosylase breaks the *N*-glycosylic bond between the 5' pyrimidine of the dimer and the corresponding sugar. The resulting AP site is then cleaved by an AP endonuclease. The selective loss of the AP endonuclease activity of the *M. luteus* enzyme relative to that of the pyrimidine dimer DNA-glycosylase on purification suggests that the two activities may be separable.

Inherently, cleavage by a DNA-glycosylase at a pyrimidine dimer requires an additional catalytic step to produce strand scission which presumably is directed by an AP endonuclease. Analysis of the mobility of the cleavage products on high resolution polyacrylamide gels suggest that *M. luteus* contains an endonuclease which acts 3' to the AP site generated by the pyrimidine dimer DNA-glycosylase. We infer that the product of cleavage by the *M. luteus* endonuclease (G-75 fraction) is a 3' AP site and a 5' thymine-thymidylate dimer, since a change in mobility of the incised fragments in β -eliminating conditions was only observed after cleavage of the 5' end-labelled and not the 3' end-labelled fragments.

The removal of the site of UV damage by excision processes in *M. luteus* must be bidirectional, both to remove a 3' sugar to provide a 3'-hydroxyl site for DNA polymerization and to excise the dimer allowing reinsertion to occur. It is of interest to note that those exonuclease activities which have been implicated in the excision process share bidirectional exonucleolytic capabilities. DNA polymerase I of *M. luteus* and *E. coli*^{3,28}, the *M. luteus* UV exonuclease²⁹, and the *E. coli* exonuclease VII³⁰, are able to initiate hydrolysis at both the 3' and 5' termini. As a consequence of this dual capability, excision is potentially a two-step process. Cleavage at AP sites may occur either 3' to the sugar as shown here, or 5' to the sugar as the result of cleavage by 5'-AP endonucleases. If cleavage occurs 5' to the sugar, then only a 5' unidirectional excision process is required for damaged nucleotide removal. It is apparent, therefore, that control of AP site incision may have a pivotal role in determining the enzymatic pathway of excision repair.

A preliminary report of some of this work has appeared elsewhere³¹. We thank R. Herriott for the preparation of yeast photolyase, and P. Seawall and P. Hanawalt for the preparation of T4 endonuclease V. W.H. is a recipient of an American Cancer Society Faculty Research Award and supported by the NCI (grant CA 26716). L.K.G. is supported by NIH training grant 5T32CA09031 and L.G. by the National Institute of General Medical Sciences (GM-22846).

Received 6 December 1979; accepted 21 March 1980.

1. Hanawalt, P. C. *Genetics* **79**, 179-197 (1975).
2. Grossman, L., Braun, A., Feldberg, R. & Mahler, I. *A. Rev. Biochem.* **44**, 19-43 (1975).
3. Hamilton, L. D. G., Mahler, I. & Grossman, L. *Biochemistry* **13**, 1886-1896 (1974).
4. Braun, A. G., Radman, M. & Grossman, L. *Biochemistry* **15**, 4006-4120 (1976).
5. Yasuda, S. & Sekiguchi, M. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1839-1845 (1970).
6. Friedberg, E. & King, J. J. *Bact.* **106**, 500-507 (1971).
7. Riazuddin, S. & Grossman, L. *J. biol. Chem.* **252**, 6280-6286 (1977).
8. Deering, R. A. & Hense, D. S. *J. Bact.* **121**, 1211-1213 (1975).
9. Van Lancker, J. A. & Tomura, T. *Biochim. biophys. Acta* **353**, 99-114 (1974).
10. Waldstein, E. A., Peller, S. & Setlow, R. B. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3746-3751 (1979).
11. Kushner, S. R., Kaplan, J. C., Ono, H. L. & Grossman, L. *Biochemistry* **10**, 3325-3334 (1971).

12. Brunk, C. F. *Nature new Biol.* **241**, 74-76 (1973).
13. Birnboim, H. C. *Photochem. Photobiol.* **22**, 71-73 (1975).
14. Setlow, R. B. & Carrier, W. L. *J. molec. Biol.* **17**, 237-254 (1966).
15. DeBoer, G., Pearson, M. L. & Johns, H. E. *J. molec. Biol.* **27**, 131-144 (1967).
16. Haseltine, W. A., Lindan, C., D'Andrea, A. & Johnsrud, L. *Meth. Enzym.* **65**, 235-248 (1980).
17. Johnsrud, L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5314-5318 (1978).
18. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
19. Riazuddin, S. & Grossman, L. *J. biol. Chem.* **252**, 6287-6293 (1977).
20. Wang, S. Y. in *Photochemistry and Photobiology of Nucleic Acids, Pyrimidine Biomolecular Products*, 296-356 (Academic, New York, 1976).
21. D'Andrea, A. D. & Haseltine, W. A. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3608-3612 (1978).
22. Carrier, W. L. & Setlow, R. B. *J. Bact.* **102**, 178-186 (1970).
23. Shaper, N. L. & Grossman, L. *Meth. Enzym.* **65**, 216-224 (1980).
24. Herriott, R. M. *Analyt. Biochem.* **95**, 77-81 (1979).
25. Rahn, R. O., Setlow, J. K. & Landry, L. C. *Photochem. Photobiol.* **18**, 39-41 (1973).
26. Lamola, A. A. *Photochem. Photobiol.* **9**, 291 (1969).
27. Kotchetkov, N. K. & Budovskii, E. I. *Organic Chemistry of Nucleic Acids*, 542-612 (Plenum, New York, 1972).
28. Kelly, R. B., Cozzarelli, L. R., Deutscher, M. P., Lehman, I. R. & Kornberg, A. *J. biol. Chem.* **245**, 39-45 (1970).
29. Kaplan, J. C., Kushner S. R. & Grossman, L. *Biochemistry* **10**, 3315-3324 (1971).
30. Chase, J. & Richardson, C. C., *J. biol. Chem.* **249**, 4553-4561 (1974).
31. Grossman, L., Riazuddin, S., Haseltine, W. A. & Lindan, C. P. *Cold Spring Harb. Symp. quant. Biol.* **43**, 947-955 (1978).
32. Birkner, K. L. & Folk, W. R. *J. biol. Chem.* **252**, 3176-3184 (1977).
33. Jacobsen, H., Klenow, H. & Overgaard-Hansen, F. *Eur. J. Biochem.* **45**, 623-627 (1974).
34. Lindahl, T. & Anderson, A. *Biochemistry* **11**, 3618-3622 (1972).

LETTERS

The triple QSO PG1115+08: another probable gravitational lens

Ray J. Weymann*, David Latham†, J. Roger P. Angel*,
Richard F. Green*, James W. Liebert*, David A.
Turnshek*, Diane E. Turnshek* & J. Anthony Tyson‡

*Steward Observatory, University of Arizona, Tucson, Arizona 85721

†Smithsonian Astrophysical Observatory, Cambridge, Massachusetts 02138

‡Bell Telephone Laboratories, Murray Hill, New Jersey 07974

Recently, Walsh, Carswell and Weymann¹ reported the discovery of two objects (0957+56A,B) with identical spectra and separated by 6 arc min. They suggested that a gravitational lens was responsible. The cumulative result of subsequent work on this object (see refs 2-9) corroborates this interpretation. The example of 0957+56A,B and the data presented here suggest a similar phenomenon is involved in the case of PG1115+08.

PG1115+08 was selected on the basis of its UV excess and identified spectroscopically as a QSO in survey work by Green and Schmidt (see ref. 10). It has a visual magnitude of 15.8 and an emission redshift of 1.722. Its 1950 coordinates are RA = 11 h 15 min 41.5 s and dec = +08° 02' 24". A finding chart is published by Green *et al.*¹¹ who report on IUE spectra showing a featureless continuum extending down to a rest wavelength of 432 Å.

In the course of an image tube spectroscopic survey at high resolution of some of the PG objects now underway at Steward Observatory by three of us (R.F.G., D.A.T. and R.J.W.), PG1115+08 was observed. The spectrum shows C IV and N V absorption at a redshift of 1.7309, slightly redward of the emission lines. Also present in absorption is a possible triplet at wavelengths of 3,923.2, 3,929.4 and 3,935.2 Å. This can be interpreted as overlapping C IV doublets at redshifts of 1.5339 and 1.5378; however, the blue member is not definitely present. Additional absorption lines occur at wavelengths of 3,968.9 Å and 4,338.4 Å. The former is possibly due to galactic H at 3,968.5 Å and the latter is unidentified. If the H line is present, the K line at 3,933.6 Å might be confusing our interpretation of the absorption 'triplet'. Although the image appears stellar on the POSS plates, observation of the object with the TV guiding monitor during a brief period of good seeing revealed two other stellar objects about 2.5 mag fainter within 3 arc s of the QSO itself.

Our next opportunity of good seeing and transparency was on 12 and 16 May, during the first trial of the MMT spectrograph. We were able to obtain spectra of the more distant of the two companions (denoted 'C') as well as the bright QSO itself (denoted 'A'). Data on the nearer companion ('B') was obtained at the Steward Observatory's 2.3-m telescope on 16 May. Figure 1 shows a polaroid print of the 2.3-m television monitor with the bright object A placed in the 2.5 arc s round aperture. Figure 2a,

b show the MMT spectra of C and A, along with the divided spectra, C/A. Figure 3 shows the corresponding data for objects A and B taken with the 2.3-m telescope. The relevant data are summarized in Table 1.

These figures and Table 1 show that to within the accuracy of the data all three spectra are identical.

However, there is a significant observational difference between 0957+56A,B and PG1115+08A,B,C: the much closer separation of the objects in PG1115+08 and the large flux ratio of A/C and A/B make the question of contamination of the spectra of C and especially B by A serious. We attempted to deal with this problem during the observations by making separate observations of blank sky with A located symmetrically opposite the slit with respect to its location during the B and C observations. The contamination is variable due to variations in the image quality. The last column of Table 1 estimates the fraction of the signal measured for B and C due to contamination by A, based on the blank sky measures. For the 2.3-m observations, the amount of scattered light was found to be direction- and colour-dependent, which produced the non-zero slope in the divided spectrum B/A as shown in Fig. 3. Observations of a

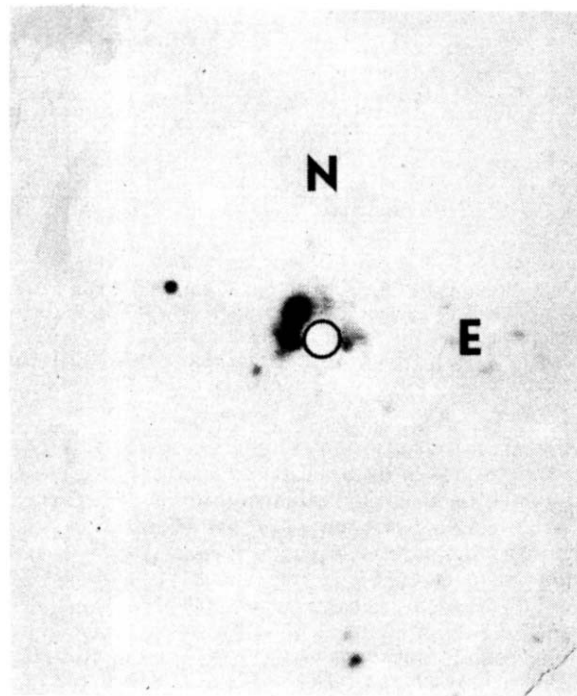


Fig. 1 Reproduction of a Polaroid photograph of the 2.3-m TV guiding screen. The brighter of the three objects, PG1115+08A was placed in the 2.5 arc s aperture (○) and the other two images, B and C, are readily visible to the west and north-west outside the aperture.

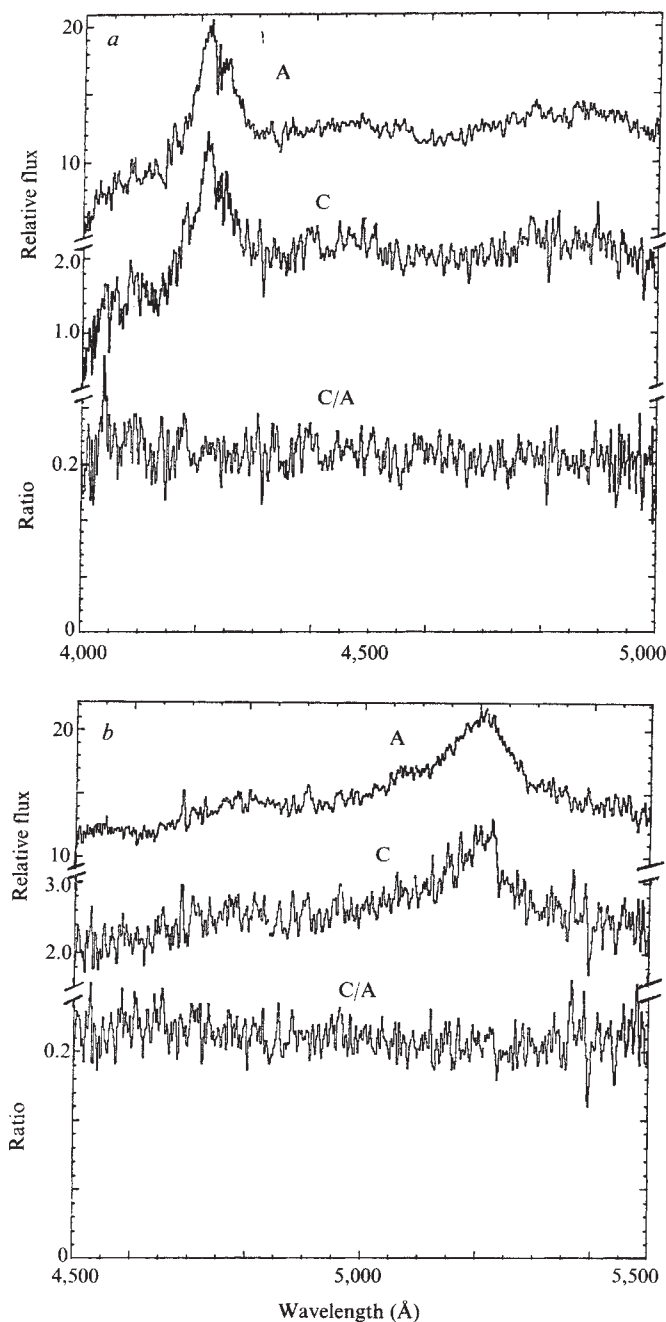


Fig. 2 Spectra of PG1115+08A and C obtained with the MMT spectrograph/intensified Reticon system. The C IV absorption features mentioned in the text are visible in both A and C just to the red of the C IV emission peak. C/A represents the result of dividing C by A.

nearby star with colours similar to the QSO provide a basis for correcting the colour of the scattered light from the blank sky measurements and also indicate that instrumental effect rather than atmospheric dispersion is the main factor in this dependence. After making this correction, the ratio B/A becomes flat to within the uncertainty.

From the divided spectra shown in Figs 2 and 3 we estimate that the difference in equivalent width of the C III] and C IV emission lines between the signal in either aperture B or C and A is $< 10\%$. This fact implies that the spectra of B and C are the same as A in the region of the emission lines to within an error of $10(1 + S/F)\%$, where S is the scattered flux from A and F is the flux from B or C. Only if 90% or more of the flux of observations B and C were scattered from A would the data be consistent with B and C not having emission lines at the same wavelengths as the C III] and C IV emission lines in A. For C the blank sky measures

Table 1 Spectroscopic data for PG1115+08

Telescope	Object	C IV $\lambda 1,549$		C III] $\lambda 1,909$		Contami- nation*
		Observed EW($\text{\AA}$)	$\lambda_{\text{OBS}}(\text{\AA})$	Observed EW($\text{\AA}$)	$\lambda_{\text{OBS}}(\text{\AA})$	
MMT†	A	60 ± 6	$4,225 \pm 5$	72 ± 6	$5,210 \pm 5$	
MMT†	C	‡	$4,225 \pm 5$	‡	$5,202 \pm 10$	0.10 ± 0.10
2.3 m§	A	—	—	67 ± 10	$5,205 \pm 10$	
2.3 m	B	—	—	‡	$5,200 \pm 10$	0.35 ± 0.10

* The contamination is the estimate of the fraction of the signal at B or C due to scattered light from A.

† The MMT spectrograph used a 3.0 by 1.5 arc min rectangular slit; the spectral resolution was 3 $\text{\AA}$ FWHM.

‡ Equivalent widths in B, C are estimated to be equal to those in A to within 10% on the basis of the divided spectra B/A, C/A.

§ The 2.3 m spectrograph used a 2.5 arc s round aperture; the spectral resolution was $\sim 10 \text{\AA}$.

Note that error estimates are subjective estimates of probable errors.

(Table 1) show that the contamination from A is probably not more than 10% and certainly not more than 20%. For object B the blank sky measures show that $\sim 35\%$ of the red light is contributed by scattering, and when corrected for colour dependence, the same ratio applies to the region of C III] emission. The possibility of B being intrinsically much redder than A and hence implying a large fraction of the signal at C III] arising from scattering seems excluded by visual observation with the TV monitor: the flux ratio of the three components seems identical through both the unfiltered camera and through an intermediate bandpass filter centred on the C III] emission line.

The data are inconsistent with B, and especially C not having significant C IV and C III] emission with wavelengths identical to A and probably identical equivalent widths as well. We thus

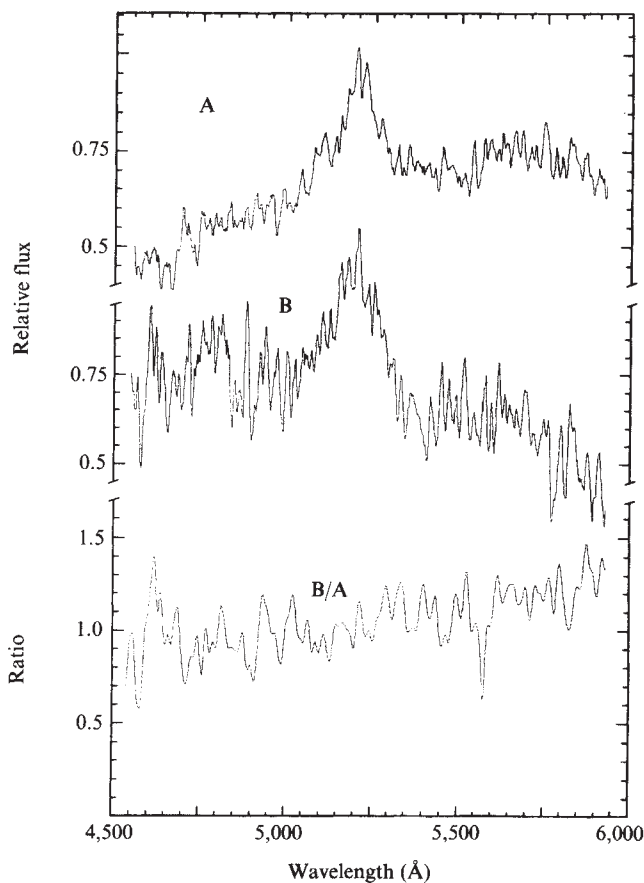
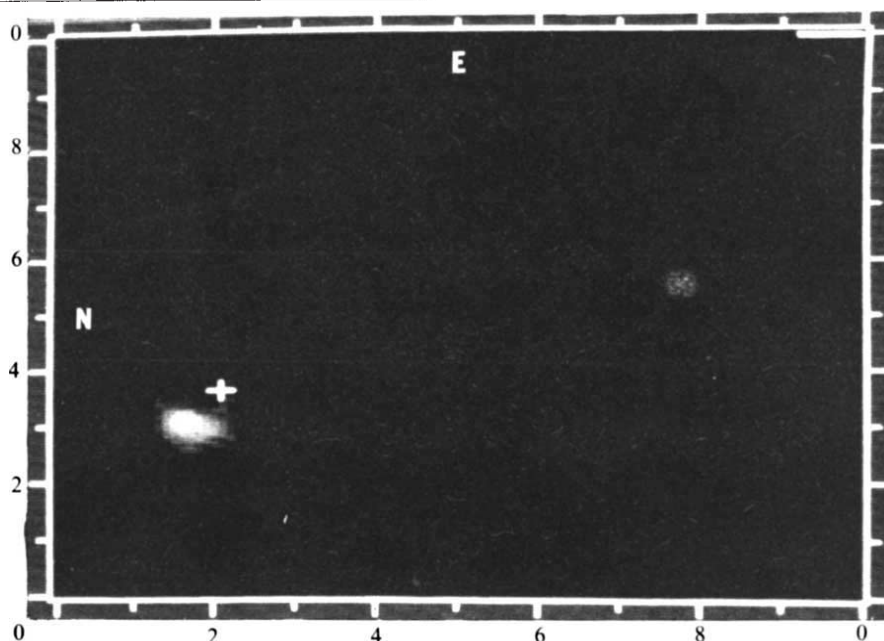


Fig. 3 Spectra of PG1115+08A and B obtained with the Steward Observatory 2.3-m telescope and Reticon spectrograph. As described in the text, colour-dependent scattering of A into the aperture during the B observation causes the divided spectra B/A not to be flat.

Fig. 4 A 20-min exposure of PG1115+08 with the Bell Labs CCD camera on the 2.3-m telescope, with seeing 2.5 arc s. The brightest of the QSO triplet, object A, has been subtracted, leaving B and C to the west and northwest and the object S 23 arc s to the south. In spite of the relatively poor seeing, relative fluxes of the three objects may be obtained by subtraction of comparison star profiles.



infer that PG1115+08 is another example of a gravitational lens.

Direct deep images of this field are of obvious interest. A CCD camera developed by one of us (J.A.T.) was attached to the 2.3-m telescope to image a 30 by 40 arc min field. The average seeing during the 20-min exposure was ~ 2.5 arc s. Figure 4 shows the result of the processed image in which the bright component A has been subtracted. This was done by combining images of nearby stars before and after the QSO exposures, scaling, moving recursively nearer the peak of the triple QSO image and subtracting. Taking $A+B+C=15.8$ mag (ref. 11), we find $B+C=18.1$ mag. The object denoted 'S' is 23 arc s to the south of the triple QSO and has a CCD magnitude about 19. Table 2 summarizes relevant data on these four images.

A second major difference between 0957+56A,B and PG1115+08A,B,C concerns the geometry of the images. There are three distinct (apparently stellar) objects readily visible and separated from each other by comparable distances. Young *et al.*⁴ and Bourassa and Kantowski¹² have shown that a rich variety of image displacements and brightness ratios are possible when there is more than one mass concentration responsible for the deflection and when realistic density distributions of matter in the deflector are taken into account. We think it premature to speculate on what combination of parameters would produce the observed image configuration until better direct images are available. We note, however, that PG1115+08 is among the most luminous QSOs and it is possible that its flux has been enhanced by the lens. Note also that the radiation from A does not pass through any clouds that are optically thick in the Lyman continuum.

Finally, we note with regret that PG1115+08 is not detectable to the 1.5-mJ flux level with VLA (see ref. 13) and hence this tool is not readily available to help in the interpretation of this fascinating object.

We thank T. Bauer, J. Geary, R. Hilliard, C. Hughes, S. Mileski, R. Miller, D. Mitchell, J. T. Williams, W. Wyatt and R. Young for exceptional efforts in completing the MMT spectrograph and detector system in time for our run and M.

Table 2 Image properties of PG1115+08A, B, C

Object	Visual Magnitude	Position angle (deg)	Separation from A (arc s)
A	15.9	—	—
B	19:	270	2.1
C	18.6	320	2.7
S	19:	161	23.0

Chabin and C. Heller for their assistance at the 2.3-m telescope. We also thank the MMT telescope operators P. Evans, W. Kindred and W. Light for making the MMT observations possible. J.R.P.A. and R.J.W. acknowledge support from the NSF. Part of the research reported here used the Multiple Mirror Telescope Observatory, a joint facility of the University of Arizona and the Smithsonian Institution. J.A.T. thanks R. Lee for help with the CCD software.

Received 28 May; accepted 4 June 1980.

- Walsh, D., Carswell, R. F. & Weymann, R. J. *Nature* **279**, 381 (1979).
- Weymann, R. J. *et al. Astrophys. J. Lett.* **233**, L43 (1980).
- Wills, B. & Wills, D. *Astrophys. J.* (in the press).
- Young, P., Gunn, J. E., Christian, J., Oke, J. B. & Westphal, J. A. *Astrophys. J.* (in the press).
- Stockton, A. (in preparation).
- Soifer, B. T. *et al. Nature* **285**, 91 (1980).
- Lebofsky, M., Rieke, G. H., Weymann, R. & Walsh, D. *Nature* **285**, 385–388 (1980).
- Greenfield, E. P., Roberts, D. H. & Burke, B. F. *Science* **208**, 495 (1980).
- Porcas, R. W., Booth, R. S., Browne, I. W. A., Walsh, D. & Wilkinson, P. N. *Nature* **282**, 385 (1980).
- Green, R. F. *Publ. A.S.P.* **88**, 665 (1980).
- Green, R. F. *et al. Astrophys. J.* (in the press).
- Bourassa, R. & Kantowski, R. *Astrophys. J.* **195**, 13 (1975).
- Shaffer, D. B., Green, R. F. & Schmidt, M. (in preparation).

Giant shells around normal elliptical galaxies

David F. Malin & David Carter

Anglo-Australian Observatory, Box 296, Epping, New South Wales 2121, Australia

Photographic enhancement of deep IIIaJ and IIIaF plates taken with the UK Schmidt and Anglo-Australian telescopes reveals the existence of giant ellipsoidal shells within and around the envelopes of several normal elliptical galaxies. The dimensions of these features are vast; they occur at radii of up to 180 kpc (assuming $H_0=50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). We report here that these features probably consist of stars and are either the result of a burst of star formation initiated by a powerful shock wave in an intergalactic medium, perhaps during the formation of the galaxies, or are old stars displaced from the nucleus by an explosive event.

The existence of very low surface brightness shells or loops around some peculiar elliptical galaxies has been reported for M89 (ref. 1), and for NGC1316 (ref. 2). We have examined apparently normal elliptical galaxies for similar features at extremely low surface brightnesses. High contrast positives of

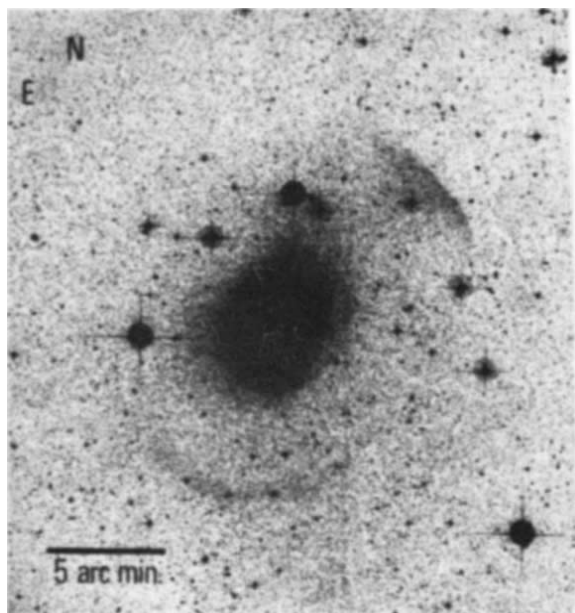


Fig. 1 Outer shells associated with NGC1344. Print made from UK Schmidt IIIaJ plate.

UK Schmidt plates were made using a contact copying process³, and low contrast features within the envelopes of the galaxies were brought out using an unsharp masking technique⁴. We found that a surprisingly high proportion of normal ellipticals showed well defined shells, or fragments of shells, at a wide range of distances from the centre of the galaxy. The shells were most clearly visible on derivatives of IIIaJ plates, which is probably an indication of the fainter limiting magnitude of this emulsion rather than of any intrinsic blueness of the shells. Shells have not previously been revealed by digital surface photometry (refs 5–8 and refs therein) owing largely to the small areas processed in these digital studies and the large angular size of the shells.

The original plates were closely examined for ghost images or internal reflections from bright stars diametrically opposite the program galaxies which might produce spurious images. None were found. Our photographic techniques have been shown¹ to be free from distracting artefacts. In one case the same shells were seen on both blue and red plates taken on different field centres. We conclude, therefore, that the features are real.

Forty-three isolated elliptical galaxies south of -18° , brighter than apparent magnitude 13 were selected from the Second Reference Catalogue¹⁰. Of this sample we examined high contrast derivatives of 12, the sole selection criterion being the ready availability of plate material from the UK Schmidt telescope. Internal or external concentric shells were found around NGC1344, NGC1395 and NGC3923 and a similar feature was seen near IC4797 but not concentric with the main body of the galaxy. A series of shells has also been noted around an SO galaxy found on direct 3.9 m AAT plates taken for another purpose. This uncatalogued object is at RA 01 h 33 min 59.0 s, dec. $-12^\circ 53' 03''$ (1950).

The most spectacular features we have found occur around the isolated elliptical galaxies NGC1344 and NGC3923, shown in Figs 1–3. A high contrast derivative of NGC1344 (Fig. 1) shows two sharp fragments of a large shell at a radius of 9 arc min (~ 56 kpc assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) from the centre of the galaxy. On the southern and western sides of the main image fragments of an inner shell (radius 2 arc min) can be seen, although this is not as well defined as the outer shell. We estimate the surface brightness of these outer shells to be about $27 \text{ mag arc s}^{-2}$, based on the appearance of the jet in M89 which has been found to have a surface brightness of

$26.9 \text{ mag arc s}^{-2}$ (Elliott and D.F.M., unpublished). Both features are just visible on examination of the original deep IIIaJ plates.

A similar print of NGC3923 shows a fragment of a giant shell in the north-east quadrant at a radius of 20 arc min (~ 180 kpc assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) and a suggestion of another fragment of the same shell in the south-west quadrant (along the major axis). Figure 2, a masked high contrast print of the inner regions of NGC3923, shows three additional distinct inner shells, lying within the envelope of the galaxy at radii of 2.2, 3.3 and 5.9 arc min. All shells are more prominent on the north or north-east sides of the galaxy and, in common with NGC1344, lie largely on the major axis. Figure 3 shows the positions of all four shells associated with this galaxy.

The shell-type galaxy found on the AAT plates is a lenticular or possibly an Sa galaxy with a high bulge-to-disk ratio, seen very nearly edge on. The major axis diameter is some 12 arcs. Two fragments of a faint shell are just visible on a normal copy, and clearly visible on a high contrast derivative; they are at a radius of ~ 25 arcs and lie near the apparent minor axis of the main body of the galaxy. On the high contrast derivatives an even fainter shell feature is visible; it lies at a radius of 54 arcs and near the minor axis of the galaxy.

In view of the extremely low contrast and surface brightness of the shells, their interpretation presents some difficulties, particularly in the absence of spectral data. We must consider the possibility that the faint shells result from reflection nebulae, caused by dust shells. Sandage⁹ gives the surface brightness (SB) of a dust reflection nebula illuminated by a galaxy as

$$\text{SB} = m - 2.5 \log [\alpha \tau / 4\pi Q (206265)^2] \text{ mag arc s}^{-2}$$

where m is the apparent magnitude of the galaxy as seen from the reflection nebula; α is the albedo of the grains, Q is the 'efficiency factor' of the grains and τ is the optical depth of the nebula. The NGC1344 outer shell is at a radius of 540 arcs; the apparent magnitude of NGC1344 is $B_t = 11.25$ (ref. 10); this gives $m = -1.66$. To cause a reflection nebula of surface brightness $27 \text{ mag arc s}^{-2}$ we would need $\alpha \tau / Q \approx 1.85$. Sandage adopts $\alpha = 0.5$; $Q = 2$ for high latitude reflection nebulae around our galaxy. If the scattering material around elliptical galaxies were assumed similar these values would imply $\tau \approx 7.4$.

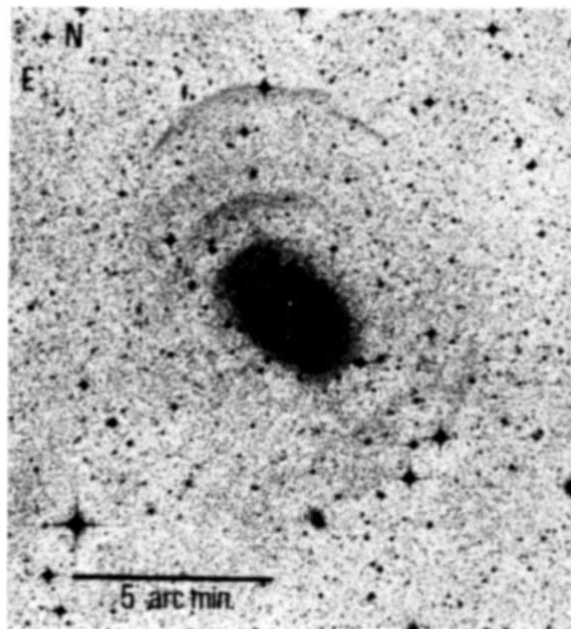


Fig. 2 Inner shells of NGC3923 print made by masking/high contrast technique from UK Schmidt IIIaJ plate.

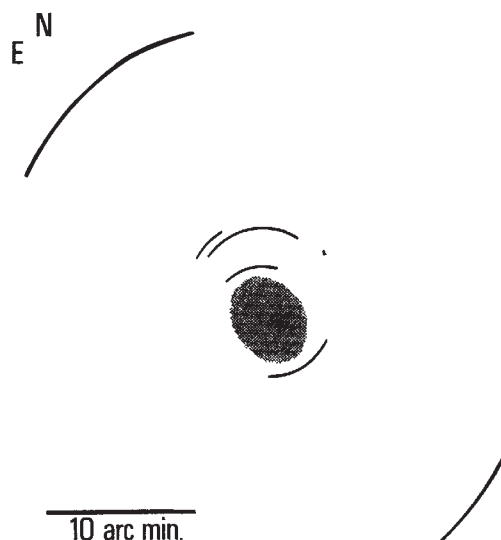


Fig. 3 Combined data from two derivatives of UK Schmidt plate of NGC3923 showing location of inner and outer shells.

We estimate a lower limit for the surface brightness of the outer (20 arc min) shell around NGC3923 to be $30 \text{ mag arc s}^{-2}$; this would imply $\alpha\tau/Q=0.5$, $\tau \approx 2$. Normal colours of these ellipticals rule out this kind of optical depth in a dust shell; but there remains the remote possibility that we are seeing reflection from a thick dust ring, which somehow contrives to resemble a thin shell. The efficiency of scattering by dust varies as λ^{-1} at optical wavelengths¹¹; therefore, one would not expect bright reflection nebulosity around a source whose light is dominated by K and M stars.

Reproductions of IIIaJ and IIIaF pairs show that the colours of the features are not much different from those of the envelopes of the galaxies. Although we cannot eliminate the possibility that they consist of emission line nebulosity the simplest interpretation is that they are stars. Although the features give the impression of being shells seen in projection we must also consider the possibility that they are weak large-scale arms—features in a plane. We believe this unlikely in NGC1344, 1395, 3923 and M89. The features around IC4797 appear more like arms, and could be tidal in origin, as there is an apparently close, disturbed, companion.

If, as seems the simplest interpretation, the features are shells of stars, then the next consideration is whether they were formed in their present location or displaced by some explosive event from the inner regions of the galaxy. It is probably significant that most examples of this phenomenon occur in isolated galaxies. The crossing time of a galaxy in a rich cluster is of the order of 10^9 yr. It would seem inevitable that a single passage through the core of a rich cluster would disrupt the observed structure. Thus the absence of such structures in rich clusters implies an age greater than 10^9 yr for the shells. Assuming a mass of $10^{12} M_{\odot}$ for NGC3923 the free fall time of the shell or ring at 180-kpc radius is 2.3×10^9 yr. This would seem to set a lower limit on the survival time of such a structure in the absence of interactions with other galaxies, and is short enough to dismiss the possibility that the shells consist of infalling primordial material.

If the shells were formed *in situ* some violent shock must have triggered a burst of star formation in an intergalactic medium which is not, or was not, as tenuous as we believe the intergalactic medium to be. Large scale shocks could be caused either by collapse, or by an explosive event in the nucleus of the galaxy. Russian cosmologists (see refs 12–14) have discussed the possibility of galaxy formation through adiabatic instabilities in the early Universe. Large scale shocks are a natural consequence of this hypothesis, but it is unclear what the present appearance of the remnants of such shocks

would be. However, if these shells are such remnants then the shocks must have contrived to place stars in stable and fairly eccentric orbits, which give rise to a surprisingly thin shell. Others (see refs 15–17) have discussed a theory of galaxy formation in which ellipticals form from mergers of spirals. M89, NGC1344 and NGC3923 exhibit no peculiarities in their colours which would lead one to suspect that they have formed from mergers of blue galaxies. Colours are not available for NGC1395. Additionally the galaxies display no irregularities in their nuclei which could be taken as an indication of cannibalism¹⁸.

We are left with the possibility that shocks, perhaps caused by an explosive event in the nucleus, formed the outer expanding shells, and that successive eruptions formed the inner structures. It is likely that the shells have existed for a substantial part of the life of the galaxy. This is supported by the fact that, apart from M89, none of the four galaxies mentioned above have active nuclei to the detection limit (12 mJy) of the 5 GHz survey of Disney and Wall¹⁹. M89, which is a compact, variable radio object has other optical peculiarities¹ which may be younger than the outer shell, 47 kpc from the nucleus. The radio properties and optical spectra of these galaxies merit further investigation.

Received 3 March; accepted 2 May 1980.

1. Malin, D. F. *Nature* **277**, 279–280 (1979).
2. Schweizer, F. *Astrophys. J.* **237**, 303–318 (1980).
3. Malin, D. F. *Nature* **276**, 591–593 (1978).
4. Malin, D. F. *Am. astr. Soc. Phot. Bull.* No. 16, 14–17 (1977).
5. King, I. R. *Astrophys. J.* **222**, 1–13 (1978).
6. Fraser, C. W. *Astr. Astrophys. Suppl.* **29**, 161–194 (1977).
7. Carter, D. & Dixon, K. L. *Astr. J.* **83**, 574–582 (1978).
8. Kormendy, J. *Astrophys. J.* **214**, 359–382 (1977).
9. Sandage, A. R. *Astr. J.* **81**, 954–957 (1976).
10. de Vaucouleurs, G., de Vaucouleurs, A. & Corwin, H. G. *Second Reference Catalogue of Bright Galaxies* (Texas Press, 1976).
11. Martin, P. G. in *Cosmic Dust*, 38 (Oxford University Press, 1978).
12. Zeldovich, Ya. B. *Proc. IAU Symp.* No. 79, 409–421 (1978).
13. Doroshkevich, A. G., Saar, E. M. & Shandarin, S. F. *Proc. IAU Symp.* No. 79, 423–425 (1978).
14. Doroshkevich, A. G., Shandarin, S. F. & Saar, E. M. *Mon. Not. R. astr. Soc.* **184**, 643–660 (1978).
15. Toomre, A. *The Evolution of Galaxies and Stellar Populations* (eds Tinsley, B. M. & Larson, R. M.) 401–416 (Yale University Press, 1977).
16. White, S. D. M. *Mon. Not. R. astr. Soc.* **184**, 185–203 (1978).
17. Aarseth, S. J. & Fall, S. M. *Astrophys. J.* **236**, 43–57 (1980).
18. Hausman, M. A. & Astriker, J. P. *Astrophys. J.* **224**, 320–336 (1978).
19. Disney, M. J. & Wall, J. V. *Mon. Not. R. astr. Soc.* **179**, 235–254 (1977).

Upper limit to any 59.35-s periodic radio emission at 18 cm from CG195.5+4.5

M. Emin Özöl

Physics Department, Middle East Technical University, Ankara, Turkey

John R. Dickel & John C. Webber

Astronomy Department, University of Illinois, Urbana, Illinois 61801

To understand the true nature of the 100-MeV cosmic γ -ray source CG195.5+4.5 (also known as γ 195+5, or Geminga), its identification at other wavelengths seems essential. Unfortunately, its positional uncertainty is large and several attempts to establish a counterpart at radio wavelengths (ref. 1 and H. A. Mayer-Hesselerwanger, personal communication), optically², in X-rays^{3,5}, and in very high energy ($>10^{11}$ eV) γ rays⁶, have produced no conclusive identification. In most cases, the reported 59-s γ -ray periodicity^{7,8} was used as a basis for a search. We report here an intensive radio search for this object at a wavelength of 18 cm using the 37-m telescope at the Vermilion River Observatory of the University of Illinois, which has a 22-arc min half-power beamwidth at this wavelength. The radiometer consisted of a parametric amplifier with a 10 MHz bandwidth operating in a noise-adding mode to give a system noise temperature of 100 K. The output was sampled and recorded at 184 ms intervals.

Observing consisted of a point-by-point search at 15-arc min intervals over a $2^\circ \times 2^\circ$ error box surrounding the γ -ray position (see Fig. 1). Each of the 99 positions was sampled for 12 min. At several positions for which there were possible signals or suggested objects from other reports, multiple observations were made. The data at each position were then binned and folded to look for a periodicity at 59.35 s, the value calculated from the P and $\dot{P}$ derived from COS B observations⁸ extrapolated to the epoch of the present observations (April–May 1979). If the adopted period is in error then, as the data are accumulated over the 12-min interval, any repeating feature will slide through the folded period and smear the apparent amplitude variation. We have divided the folded data into 25 bins which allows us to cover an unsmeared range in a period of 200 ms, well within the published uncertainty. After folding, the resultant pulse amplitude was evaluated for a random occurrence probability by a χ^2 analysis. The average r.m.s. noise per bin in this analysis was:

$$\sigma = 0.015 K(T_A) = 0.075 \text{ Jy} \quad (1)$$

In the distribution of the largest deviation from the mean at each position no deviations greater than 5σ were found and none are statistically significant. We can, therefore, place an upper limit on any 59.35-s periodic emission with a duration of $(59.35/25) = 2.37$ s at this 5σ level of

$$F(18\text{cm}) < 0.375 \text{ Jy} \quad (2)$$

Any periodic signal with a duration longer than one bin will be correlated in adjacent bins increasing the effective integration time to allow detection to a smaller limit by a factor of $(2.37/\text{duration})^{1/2}$. Shorter pulses will apparently be spread over the whole 2.37-s interval and would, therefore, have correspondingly higher detection limits.

A compilation of measurements as well as upper limits to the flux density of CG195.5+4.5 at different photon energies, including the present results, is given as Fig. 2. By comparison with the spectrum of the Crab pulsar, one might conclude that the object could be a weak old pulsar; however, it is difficult to reconcile a 59-s period with the spin of a standard pulsar. If the period difficulty can be overcome, the reported γ -ray pulse profile^{7,8} with multiple (probably four) peaks is suggestive of pulsar emission models with two pulses per pole⁹. Such objects

have not been observed, but they may be a subclass of the γ -ray sources reported by the COS B group^{10,11}. A search for periodic emission from such γ -ray sources would prove very useful in this respect.

The reported γ -ray periodicity may possibly be false, because in each of the γ -ray reports^{7,8} the amplitude of the pulse profile is less than 4σ . If the emission is not pulsed, other source models such as a dark cloud complex excited by a nearby supernova¹²; a system containing a compact object (a close binary or black hole), or a special kind of supernova remnant (SNOB)¹³ could be considered as possible explanations for the γ -ray emission from this region of space.

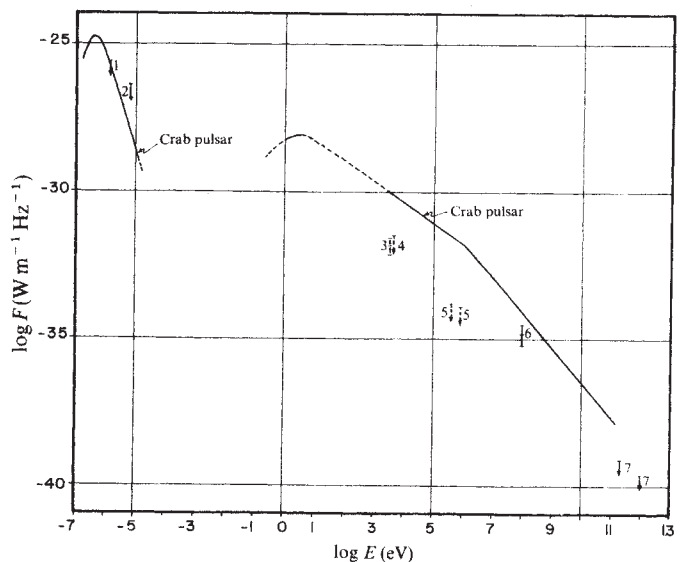


Fig. 2 An energy spectrum of CG195.5+4.5 compiled from all reported observations. Points with broken lines represent data for which no periodicity search at the γ -ray period was made. For comparison, the energy spectrum of the Crab pulsar¹⁹ is shown. 1, Ref. 1, 325 MHz; 2, present study, 1,666 MHz; 3, ref. 3, 2–10 keV, Uhuru; 4, ref. 4, 2–6 keV, HEAO 1; 5, Coe *et al.* (1978), ~ 500 keV, Ariel 5; 6, Hermesen *et al.* (1977), ~ 100 MeV, COS B; 7, ref. 6, > 200 GeV.

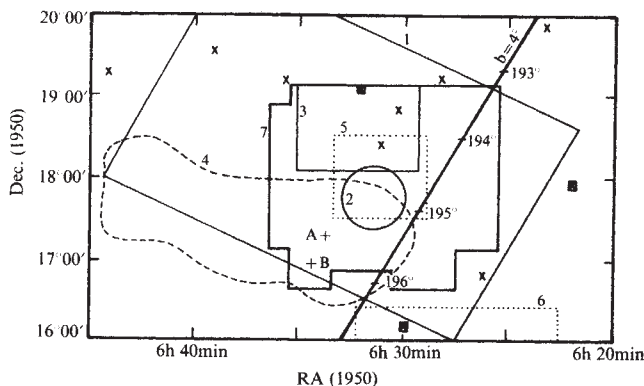


Fig. 1 The region of the sky covered by the present survey together with other reported observations for CG195.5+4.5. The numbers on the various boundaries are for: 1, the SAS 2 error box⁷; 2, the reduced COS B error box (R. Lamb, personal communication); 3, the HEAO 1 90% error box⁴; 4, the Uhuru 2σ X-ray contour where A and B are the most probable source positions³; 5, Bonn 18-cm survey (H. A. Mayer-Hesselwander, personal communication); 6, Westerbork 49-cm survey¹⁸; 7, the present survey at 18 cm. Continuum radio sources previously catalogued in refs^{14–18} are indicated by X. Those found in the present survey are enclosed in boxes.

Several continuum surveys at long radio wavelengths have covered all or part of this area^{14–17} and 13 point sources have been catalogued within the boundaries of Fig. 1. No source lies particularly near the best position for the γ -ray source and all appear to have 'normal' spectra. To check for continuous radio emission we have mapped a large area around the source at 18 cm using the same telescope system described above. This is the shortest wavelength at which the region has been surveyed. Scans in right ascension have been combined to provide a 20-s integration time for each half-beamwidth interval. A slight additional noise was added to the system by the scanning procedure to give an r.m.s. noise of 0.15 Jy on the map. Only three previously catalogued sources were expected to have large enough flux densities to be seen at our flux density limit at the frequency of the present survey. These three were found as expected but no other sources brighter than our 5σ limit of 0.75 Jy were detected. Thus any source with a peculiar spectrum must be weak and no strong candidate for the γ -ray source is apparent.

We thank K. S. Yang, J. B. Oder and G. Whittaker for maintenance of the telescope systems. This research was initiated under a Fulbright Intercountry Exchange Visit and is supported by NATO research grant RG1680. J.C.W. is supported by the NSF. Computer time was provided by the University of Illinois Research Board.

Received 31 December 1979; accepted 15 April 1980.

1. Mandolesi, N., Morigi, C. & Sironi, G. *Astr. Astrophys.* **67**, L5 (1978).
2. van den Bergh, S. *Astr. J.* **84**, 71 (1979).
3. Julien, P. F. & Helmken, H. F. *Nature* **272**, 699 (1978).
4. Lamb, R. & Worral, D. *Astrophys. J. Lett.* **231**, L121 (1979).
5. Haymes, C., Meegan, C. A. & Fishman, G. J. *Astro. Astrophys.* **79**, 88 (1979).
6. Helmken, H. F. & Weeks, T. C. *Astrophys. J.* **228**, 531 (1979).
7. Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. *Astrophys. J.* **213**, 252 (1977).
8. Masnou, J. L. *et al.* *12th ESLAB Symp.*, ESA-SP-124, 33 (1978).
9. Cheng, A. & Ruderman, M. Preprint Rutgers Univ. (1979).
10. Swanenburg, B. N. *Europhys. Study Conf.: γ -ray Astronomy after COS B*, Erice (1979).
11. Pinkau, K. *Nature* **277**, 17 (1979).
12. Abdulvahap, M. & Morrison, P. *Astrophys. J. Lett.* **221**, L33 (1978).
13. Montmerle, T. *Astrophys. J.* **231**, 95 (1979).
14. Pauliny-Toth, I. I. K., Wade, C. M. & Heesch, D. S. *Astrophys. J. Suppl.* **13**, 65, (1966).
15. Dickel, J. R., Yang, K. S., McVittie, G. C. & Swenson, G. W. *Astrophys. J.* **72**, 757 (1967).
16. Hoglund, B. *Astrophys. J. Suppl.* **15**, 61 (1967).
17. Gower, J., Scott, P. F. & Wills, D. *Mon. Not. R. astr. Soc.* **71**, 49, (1967).
18. Bignami, G. F., Gavazzi, G. & Harten, R. H. *Astr. Astrophys.* **54**, 951 (1977).
19. Manchester, R. N., Taylor, J. H. *Pulsars*, 59 (W. H. Freeman, San Francisco, 1978).

Does feroxyhyte occur on the surface of Mars?

Roger G. Burns

Department of Earth and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The red colour of Mars has long been attributed to the occurrence of ferric oxides on its surface and in atmospheric dust storms. Such a belief has been accentuated by results of Viking Lander experiments on the surface of Mars, which suggested the presence of maghaemite, γ -Fe₂O₃ (refs 1, 2). This dark brown, ferromagnetic iron (III) oxide phase not only conforms with the colour and magnetic properties of dust on the martian surface, but suggests that maghaemite acted as a catalyst in some of the biological experiments performed *in situ* on Mars³. Many of the physical properties and chemical reactions attributed to maghaemite, however, are also displayed by δ -FeOOH, an oxide hydroxide polymorph of ferric iron^{4,5}. On Earth, δ -FeOOH occurs as the mineral feroxyhyte in gleyed soils and in submarine manganese nodule deposits⁶. Some of the properties and paragenetic relationships of feroxyhyte are described here and it is suggested that δ -FeOOH is a prime candidate for the red-brown ferromagnetic phase coating the surface of Mars.

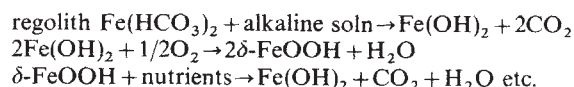
Synthetic δ -FeOOH is formed as a deep-brown precipitate during the oxidation of Fe(OH)₂ in partially deoxygenated, slightly alkaline solutions^{7,8}. It is generally poorly crystalline and may contain up to 3 wt % excess H₂O (ref. 7). Significantly, δ -FeOOH differs from other FeOOH polymorphs by being ferromagnetic ($T_c \approx 450$ K), and therefore resembles γ -Fe₂O₃. Depending on its particle size, the saturation magnetization of δ -FeOOH is 30–50% the value of γ -Fe₂O₃ at ambient martian temperatures (200 K)^{7,9}, so that δ -FeOOH, too, will adhere to magnets such as those attached to the Viking Lander spacecraft¹. The IR spectrum of δ -FeOOH contains three maxima in the range 2.95–3.4 μ m (refs 7, 10); similar spectral features are observed in remote-sensed spectra of Mars¹¹. The catalytic efficiency of δ -FeOOH in the decomposition of H₂O₂, for example, is 10-fold higher than α -FeOOH (goethite) and α -Fe₂O₃ (haematite)¹², and is comparable to γ -Fe₂O₃ (maghaemite) which was postulated to interfere with the biological experiments on the Viking Landers³.

In the marine environment on Earth, the formation of feroxyhyte is believed to involve redox reactions in buried sediments^{6,13}. The Fe(HCO₃)₂ generated there migrates in

pore waters to the surface of the sediments. At the sediment-seawater interface, where the temperature and pH are typically 275 K and 8.1, respectively, Fe(OH)₂ is formed initially, but is oxidized by the oxygenated seawater to feroxyhyte. This poorly crystalline δ -FeOOH phase coats debris and authigenic silicates such as zeolites, and forms a substrate for the deposition of manganese (IV) oxides in ferromanganese crust and nodule deposits¹⁴. The δ -FeOOH phase is also susceptible to secondary redox reactions inside manganese nodules, where it catalyses the decomposition of enclosed biogenic debris and organically chelated Ni(II) and Cu(II) species¹⁵, leading to the enrichment of these transition metals in manganese nodules on the sea floor beneath equatorial high biological productivity zones.

Such processes in the terrestrial marine environment enables us to postulate on the reactions on Mars which might lead to the formation of feroxyhyte in the martian regolith. Ferrous ions, derived from disintegration of basalt by chemical weathering, mechanical abrasion (ablation and freeze-thaw) and photochemical¹⁶ processes, form stable Fe(HCO₃)₂ in the chloride-sulphate-rich, CO₂-saturated, O₂-depleted brines in the permafrost on Mars. When the Fe(HCO₃)₂ is slowly oxidized, poorly crystalline feroxyhyte is produced which forms a thin red-brown veneer on the fractured rock surfaces. The fine-grained feroxyhyte particles are easily transported during dust storms and become major components in the bright areas of the martian surface. The large specific surface of the feroxyhyte particles also makes them effective substrates for the reversible chemisorption of water vapour from the martian atmosphere.

The synthesis and chemical properties of δ -FeOOH might also explain some of the results obtained during the Viking biological experiments on Mars^{3,17}. Metal peroxides or superoxides on the martian surface when moistened would liberate oxygen and produce alkaline solutions, thereby initiating the following reactions:



The biological experiments on Mars thus promoted the conversion of Fe(HCO₃)₂ in the regolith to feroxyhyte, the catalytic properties of which led to the breakdown of nutrients supplied during the Viking Lander experiments.

Feroxyhyte, therefore, has suitable colour, magnetic, chemisorption, spectral, redox and paragenetic properties to make it a constituent of the surface of Mars.

This research was supported by the NSF grant OCE 78-27495; and by NASA grant NSG 7604.

Received 20 February; accepted 14 April 1980.

1. Hargraves, R. B., Collinson, D. W., Arvidson, R. E. & Spitzer, C. R. *J. geophys. Res.* **82**, 4547 (1977).
2. Toulmin, P. III *et al.* *J. geophys. Res.* **82**, 4625 (1977).
3. Oyama, V. I. & Berdahl, B. J. *J. geophys. Res.* **82**, 4669 (1977).
4. Burns, R. G. & Burns, V. M. in *Marine Manganese Deposits* (ed. Glasby, G. P.) 212 (Elsevier, Amsterdam, 1977).
5. Murray, J. W. in *Marine Minerals* Ch. 2 (ed. Burns, R. G.) 47 (Mineralogy Society of America Monograph 6, 1979).
6. Chukhrov, F. V., Zvyagin, B. B., Yermilova, L. P. & Gorshkov, A. I. *Miner. Deposita* **11**, 24 (1976).
7. Okamoto, S. J. *Am. ceram. Soc.* **51**, 594 (1968).
8. Atkinson, R. J. *Aust. J. Chem.* **29**, 2149 (1976).
9. Coey, J. M. D. & Khalafalla, D. *Phys. Stat. Sol. A* **11**, 229 (1972).
10. Feitknecht, W., Haei, H. & Dvorak, V. in *Proc. 6th int. Symp. Reactivity of Solids*, 237 (1969).
11. Houck, J. R., Pollack, J. B., Sagan, C., Schaack, D. & Decker, J. A. Jr *Icarus* **18**, 470 (1973).
12. Sara, J. *Chem. Listy*, **63**, 112 (1969).
13. Burns, R. G. & Burns, V. M. in *The Sea* Vol. 7 (ed. Emiliani, C.) (Wiley, New York, in the press).
14. Burns, R. G. & Burns, V. M. *Nature* **255**, 130 (1975).
15. Burns, V. M. & Burns, R. G. *Earth planet. Sci. Lett.* **39**, 341 (1978).
16. Huguenin, R. L., Prinn, R. G. & Maderazzo, M. *Icarus* **32**, 270 (1977).
17. Klein, H. P. *Icarus* **34**, 666 (1978).

Instantaneous structure of an MgSiO₃ melt simulated by molecular dynamics

Yoshito Matsui

Institute for Thermal Spring Research, Okayama University, Misasa, Tottori-ken 682-02, Japan

Katsuyuki Kawamura

Geological Institute, Faculty of Science, The University of Tokyo, Hongo, Tokyo 113, Japan

A series of molecular dynamics (MD) calculations¹ has been carried out to visualize the atomic configuration in MgSiO₃ melt and glass. The 'computer-generated' configuration seemed to be realistic. At zero pressure the Si–O–Si angle distribution gave a clear peak at ~145° in agreement with the result of ordinary X-ray study on vitreous SiO₂ (ref. 2). Coordination of O around Mg was irregular, because the whole structure is dominated by packing of the rather regular SiO₄ tetrahedra which are linked together by sharing O atoms at corners. The 'melt' also showed a partial conversion from a 4- to 6-coordination state on 'compression' within a reasonable density range, accompanied by a steady decrease in the Si–O–Si angles. The MD calculation, which is simply the numerical integration of the classical newtonian equations of motion for many particles in a hypothetical periodic space, has been successfully applied to various molten ionic salt systems¹. Following Woodcock *et al.*'s³ first application of MD to vitreous SiO₂, we decided to extend the MD calculation to systems of geochemical importance and report our results here.

To carry out the MD calculation, a set of potential parameters is needed to evaluate the force acting on every ion. Recently, Miyamoto and Takeda (personal communication) estimated the parameters for Si⁴⁺ and O²⁻ in the following pair potential expression assuming a purely ionic nature of the system:

$$\phi_{ij} = \frac{z_i z_j e^2}{r_{ij}} + f(b_i + b_j) \exp\left(-\frac{a_i + a_j - r_{ij}}{b_i + b_j}\right)$$

where ϕ_{ij} is the pair potential between ions *i* and *j*, *z* denotes the formal charge number, *e* is the unit charge, *r_{ij}* is distance

between *i* and *j*, *a_i* and *b_i* are crystal radius and softness parameter of ion *i*, and *f* is an arbitrary constant. Miyamoto and Takeda refined the relevant parameters using the WMIN program written by Busing⁴, to minimise the lattice energy of Mg₂SiO₄ (forsterite) with structural parameters given by Smyth and Hazen⁵, taking *a_{Mg}* = 0.97 Å (1 Å = 100 pm) and *b_{Mg}* = 0.065 Å given by Busing⁴ and *f* = 1 kcal mol⁻¹ Å⁻¹ (= 6.9478 × 10⁻⁶ dyn) as fixed values.

The 215 ions (43 MgSiO₃) were placed into a cubic basic cell (edge length, *L*) with randomly generated coordinates and an assumed zero pressure density 2.49 g cm⁻³ (*L* = 14.225 Å), which corresponds to a 20% increase in volume over MgSiO₃ orthopyroxene (31.4 cm³ mol⁻¹) (ref. 6). In the following calculation the summation of repulsive force and the first (direct lattice) series in the Ewald sum of electrostatic force were terminated at 0.5*L*, and the second (reciprocal lattice) series in the Ewald sum was terminated within 61 adjacent unit cells. The relative error due to the termination was estimated to be within 1%.

The MD calculation was started at constant temperature (8,000 K) with a time increment 10⁻¹⁶ s. On approaching thermal equilibrium the calculation was switched to a constant energy mode with a time increment 1.25 × 10⁻¹⁵ s. After disappearance of systematic drift in temperature, additional 750 steps were calculated at constant energy. The 'product' was then 'cooled' at a rate of 100 K per 10 steps down to 2,000 K, and annealed at ~2,000 K for more than 1,000 steps and further quenched to 500 K when necessary.

The 'compression experiments' were carried out starting from the configuration obtained above, decreasing interatomic distances uniformly to give 0.8 *V*₀ (3.11 g cm⁻³), 0.7 *V*₀ (3.56 g cm⁻³) and 0.6 *V*₀ (4.15 g cm⁻³). The same procedure was applied in subsequent computation as outlined above.

The calculated structures for *V*₀ and 0.8 *V*₀ were similar with each other. A typical instantaneous configuration of ions for 0.8 *V*₀ at ~500 K is shown in Fig. 1 as a stereoscopic pair. The important features are as following. First, regular SiO₄ tetrahedra show an extensive polymerization by sharing O ions at corners: out of four O ions per one tetrahedron 2.2–2.3 O ions are shared by adjacent tetrahedra on average. As Fig. 1 shows, however, this does not imply the existence of the infinite SiO₃ chains as those in pyroxenes. Second, the distribution of Si–O(bridging)–O angle shows a clear peak at ~145° as stated earlier. Third, coordination of O ions around Mg is irregular, the number of the nearest O ions ranging from three to four.

A striking change in configuration is observed at 0.6 *V*₀ as can be seen in Fig. 2. At least one-third of Si atoms are more or less regularly coordinated by six O ions. Edge sharing among the adjacent SiO₆ octahedra, which is common in the crystals containing Si in 6 coordination, is extensive although this is not

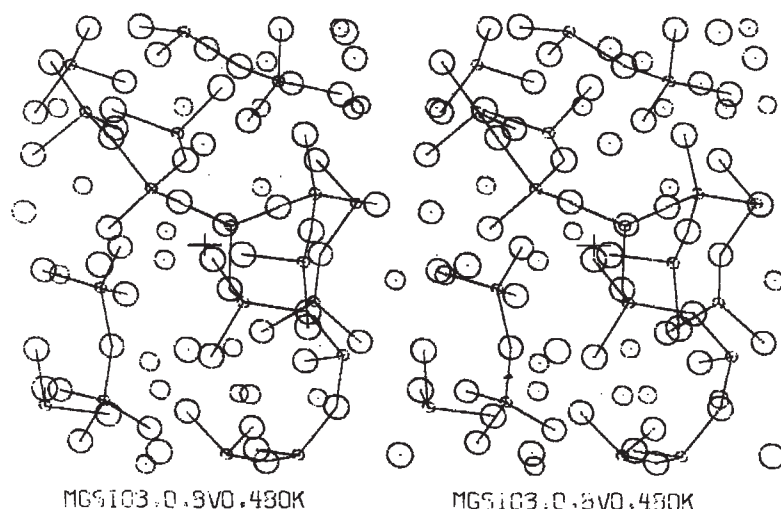
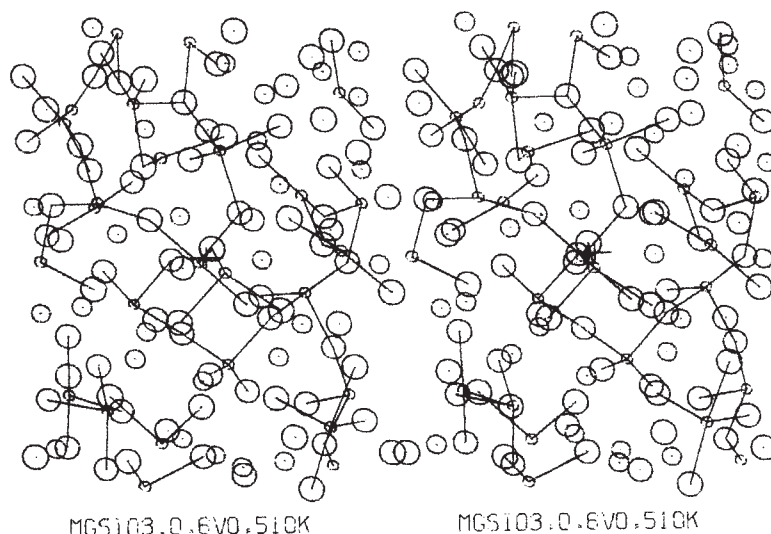


Fig. 1 Stereoscopic pair drawing of the instantaneous structure of MgSiO₃ at 3.11 g cm⁻³ and 480 K. The Si–O pairs with distances less than 179 pm are connected by solid lines. Circles represent Si, Mg and O atoms in the increasing order in sizes.

Fig. 2 Stereoscopic pair drawing of the instantaneous structure of MgSiO_3 at 4.15 g cm^{-3} and 510 K. The Si-O pairs with distances less than 175 pm are connected by solid lines. Regular SiO_6 octahedra are seen at centre and lower right.



clear in Fig. 2 because of the overcrowded population of ions. The coordination number of Mg, too, increases on 'compression': Most of Mg ions are in 4-6 coordination with respect to the nearest O ions.

In contrast to the remarkable change in configuration, calculated radial distribution functions (RDF) displayed rather little variation: The first maxima for Si-O, Mg-O and O-O distances shifted from 1.62 to 1.66 Å, 1.96 to 1.92 Å (a decrease) and from 2.65 to 2.51 Å, respectively, on compression. In view of the broadening of peaks in RDF on compression, however, this does not necessarily mean a decrease in the mean Mg-O distance. The small change in RDF might have been a reason for the pessimistic conclusion by Woodcock *et al.*³ that most Si atoms remain in 4 coordination even under a heavy 'compaction'.

The crystalline MgSiO_3 is known to assume the ilmenite structure under pressures above 24 GPa at 1,000 °C where Si atoms are in 6 coordination with a density 3.795 g cm^{-3} (at ambient conditions)⁷ and further transforms to the orthorhombic perovskite structure (4.108 g cm^{-3} at ambient conditions) at 27 GPa and 1,000 °C (refs 8, 9). Note that the simulated melt or glass undergoes a similar mode of coordination change in a comparable density range with the solid-solid transitions, although in the molten or vitreous state the change seems to be gradual.

The MD calculation provide a very promising way of obtaining information of both the structural and physical properties even in the not-so-perfectly ionic systems as silicate melts, of which knowledge in the atomistic level has been recognized to be vital in recent years^{10,11}.

We thank Dr I. Okada for instruction and computer programs, Dr H. Takeda and Mr M. Miyamoto for permission to use potential parameters before publication, and Professor W. S. MacKenzie and Dr M. J. Dempsey for valuable comments.

Oceanic bathymetry profiles flattened by radiogenic heating in a convecting mantle

Gary T. Jarvis & W. R. Peltier

Department of Physics, University of Toronto, Toronto, Ontario, Canada M5S 1A7

Reliable measurements of both ocean floor topography and heat flow indicate a linear dependence on the square root of the age, t , of the ocean floor for $t \leq 90$ Myr (ref. 1) or about half the age of the large oceanic plates. Consequently, major topographic and heat flow variations perpendicular to mid-ocean ridge axes are generally believed to be thermally induced and associated with contraction of the lithosphere as it moves away from the ridges¹⁻⁴. For $t > 90$ Myr, measurements of bathymetry (but not of heat flow) indicate a systematic departure from a $t^{1/2}$ relationship reaching almost 20% at the oldest ages. We present here numerical calculations of thermally induced topography above two-dimensional convection cells which are heated partially from within and partially from below. These exhibit the same general features as oceanic bathymetry and we suggest that departures of the latter from a $t^{1/2}$ dependence may be due to radiogenic heating within the mantle.

Ignoring horizontal conduction and assuming isostatic compensation at the base of the lithospheric plate, Parsons and McKenzie² derived the following energy balance equation for a column through the plate:

$$-(\rho_m - \rho_w) \frac{C_p}{\alpha} \frac{\partial D}{\partial t} + q_u = q_b + \int_z H'(z) dz \quad (1)$$

where ρ_m is the mantle density (below the plate), ρ_w is the density of water, α is the coefficient of thermal expansion, C_p is the specific heat, D is the depth of the ocean floor beneath the sea surface, t is the time, q_u is the heat flux across the upper surface of the plate, q_b is the heat flux into the base of the plate, H' is the rate of radioactive heating per unit volume in the column and the integral is taken over the height, z , of the column. Note that any thermal models with the same total heat sources given by the right-hand side of equation (1) will predict the same temporal variations of D and q_u . If there are no heat sources on the right-hand side, equation (1) reduces to

Received 28 February; accepted 6 May 1980.

1. Woodcock, L. V. *Advances in Molten Salt Chemistry* (eds Braunstein, J. *et al.*) 1-74 (Plenum, New York, 1975).
2. Mozz, R. L. & Warren, B. E. *J. appl. Crystallogr.* **2**, 164-172 (1969).
3. Woodcock, L. V., Angell, C. A. & Cheeseman, P. *J. chem. Phys.* **65**, 1565-1577 (1976).
4. Busing, W. R. *Trans. Am. Crystallogr. Ass.* **6**, 57-72 (1970); *J. chem. Phys.* **57**, 3008-3009 (1972).
5. Smyth, J. R. & Hazen, R. M. *Am. Miner.* **58**, 588-593 (1973).
6. Stephenson, D. A., Sclar, C. B. & Smith, J. V. *Miner. Mag.* **35**, 838-846 (1966).
7. Ito, E. & Matsui, Y. *High-Pressure Research—Applications in Geophysics* (eds Manghnani, H. & Akimoto, S.) 439-461. (Academic, New York, 1977).
8. Ito, E. & Matsui, Y. *Earth planet. Sci. Lett.* **38**, 443-450 (1978).
9. Yagi, T., Mao, H. & Bell, P. M. *Phys. Chem. Miner.* **3**, 97-100 (1978).
10. Henderson, P. *Miner. Mag.* **43**, 399-404 (1979).
11. Kushiro, I. *Earth planet. Sci. Lett.* **41**, 87-90 (1978).

that governing the boundary layer behaviour for high Rayleigh number viscous fluids heated entirely from below⁵⁻⁹. Away from the vertical plumes, the surface heat flow is primarily due to conductive cooling of the initially hot plume material moving horizontally across the upper surface. Balancing the horizontal advection and vertical diffusion, Turcotte and Oxburgh⁵ first predicted that $q_u \propto t^{-1/2}$; hence from equation (1) $D \propto t^{1/2}$. Although this prediction is borne out for $t \leq 90$ Myr, at later times D increases more slowly than predicted and a non-zero heat source term can be inferred for $t > 90$ Myr.

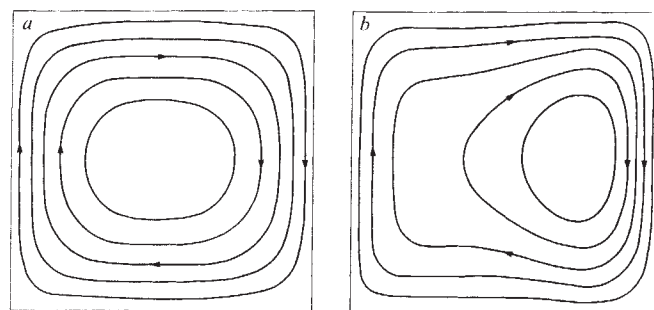


Fig. 1 Numerically determined streamlines of the flow in convection cells which are: *a*, heated entirely from below; and *b*, heated 20% from within and 80% from below. Both solutions shown here were obtained on a 96×96 finite-difference grid at $R = 1.25 \times 10^7$. The main feature is the different orientation of near-surface streamlines in the two cases. The asymmetry of the flow structure in *b* results in advective heat transport towards the upper surface for a major portion of the cell.

Parsons and McKenzie² have reviewed several models which include a variety of heat sources in an attempt to account for this 'flattening' of the bathymetry profile with respect to the $t^{1/2}$ behaviour predicted by boundary layer theory. We consider here another source of basal heating, q_b , which in the case of whole mantle convection may account for the observed ocean floor flattening. This is the vertical advection of heat into the upper thermal boundary layer which occurs continuously across convection cells which are heated partially from within and partially from below. We assess this mechanism quantitatively using high Rayleigh number model solutions obtained numerically on two-dimensional finite-difference grids. Although direct application to the Earth's mantle is somewhat speculative, the basic physics is not and has apparently been overlooked previously.

The numerical scheme used is essentially the same as that used by McKenzie *et al.*¹⁰ and Jarvis and McKenzie¹¹ except that the program package for solving Poisson's equation has been replaced by a more flexible (though less efficient) package available from NCAR (National Centre for Atmospheric Research). The solutions discussed below were obtained on finite-difference grids of various dimensions ranging from 24×24 to 96×96 mesh points. Free-slip boundary conditions were imposed together with a constant temperature at the upper surface and a constant heat flux at the base. Solutions were obtained in square boxes with vertical boundaries being planes of mirror symmetry with respect to the temperature and velocity fields. The Boussinesq approximation was assumed and thermodynamic variables were assumed constant.

The two dimensionless parameters which are relevant to the following discussion are the Rayleigh number

$$R = \frac{g\alpha d^4 E}{C_p \kappa^2 \eta} \quad (2)$$

and the ratio of internal heating to total heat flow across the upper surface of the box

$$\mu = Hd/(F + Hd) \quad (3)$$

where g is the acceleration due to gravity, d is the depth of the convection cell, E is the heat flux across the top of the box, κ is thermal diffusivity, η is the viscosity, F is the heat flux into the base of the box, H is the rate of radioactive heating per unit volume, and other parameters are as defined above (note that $E = F + Hd$).

Several numerical models were run varying both R and μ to study the dependence of the bathymetry D on these parameters. We find that flattening of the bathymetry occurs as a consequence of the shift of the centre of circulation towards the descending plume in partially internally heated flows (Fig. 1). The broad zone of upwelling produced by this shift can result in advective heat transport to the base of the thermal boundary layer well beyond the mid-point of the box.

The bathymetry was computed using a simple boundary layer expression¹² which is consistent with equation (1),

$$D(x) = \frac{\alpha \rho_m}{\rho_m - \rho_w} \int_0^{z_1} (T_m - T(x, z)) dz \quad (4)$$

where ρ_m is the mantle density, ρ_w is the density of water, T_m is the temperature in the central core, z is the vertical coordinate measured positively downward and z_1 is the depth of isostatic compensation (taken to be immediately below the thermal boundary layer). Although $D(x)$ can be expressed more accurately in terms of the dynamic forces associated with the fluid flow^{10,14}, the conclusions presented below are not

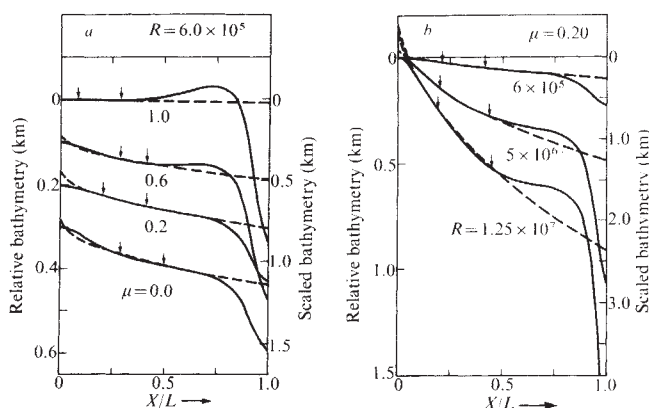


Fig. 2 Plots of thermally induced bathymetric variation across the top of convection cells of width L . Bathymetry computed from the model solutions is indicated by the solid lines. Reference curves, proportional to $x^{1/2}$ and constrained to fit the model curves at the points indicated by the small arrows, are shown as broken lines. Bathymetry is plotted relative to the highest point. In *a*, $R = 6.0 \times 10^5$ and μ varies from 0.0 to 1.0. Successive curves have been offset by 0.1 km of relative bathymetry at $x=0$ for the sake of clarity. All model solutions for *a* were obtained on a 24×24 finite-difference grid. In *b*, $\mu = 0.20$ and R varies. For $R = 6 \times 10^5$, 5×10^6 and 1.25×10^7 , the respective finite-difference grids had dimensions of 24×24 , 64×64 , and 96×96 . The left-hand vertical axes indicate the bathymetry as computed using mean mantle values for ρ_m and α (as given in the text). The right-hand vertical axes indicate the bathymetry corrected for near surface values of these parameters (3.33 g cm^{-3} and $3.2 \times 10^{-5} \text{ K}^{-1}$ respectively¹).

very sensitive to which equation is used (this point will be discussed in more detail elsewhere).

The dependence of $D(x)$ on both μ and R is illustrated in Fig. 2. The sequence of bathymetry profiles at $R=6 \times 10^5$ (Fig. 2a) shows that $D(x)$ departs from the reference $x^{\frac{1}{2}}$ curves increasingly as μ increases. We define the percentage flattening, P , as the maximum difference between the model curve and the reference $x^{\frac{1}{2}}$ curve expressed as a percentage of the value on the reference curve; P increases with μ . The sequence of bathymetry profiles at $\mu=0.20$ (Fig. 2b) shows that P is also an increasing function of R (for $R=6 \times 10^5$, 5×10^6 and 1.25×10^7 , $P=1$, 12 and 19% respectively).

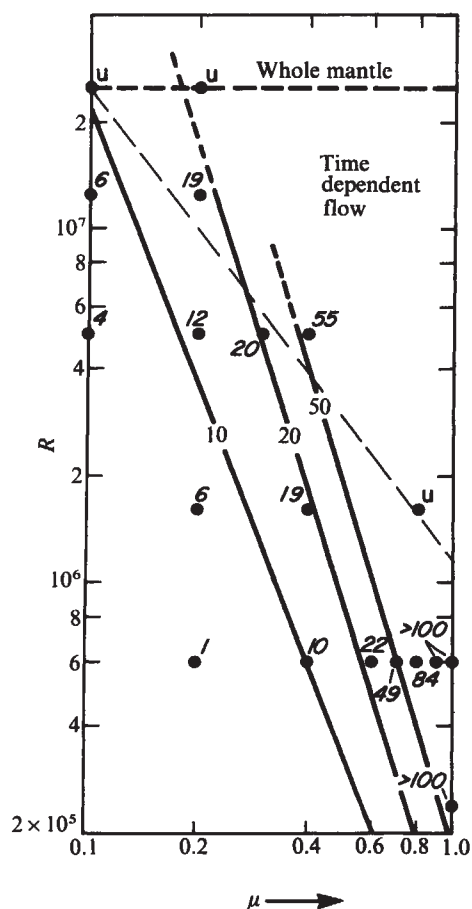


Fig. 3 Domain diagram on which the percentage flattening P is plotted as a function of R and μ . Solid circles indicate the (R, μ) coordinates for which values of P were obtained and the adjacent numbers give the values of P (%). Contours of constant P are shown for $P=10$, 20 and 50%. The broken diagonal line roughly divides domains of steady and non-steady single cell flows: Steady single-cell solutions can be obtained at all points below the line, time-dependent (or multi-cell) solutions above. Points for which solutions were too unsteady to compute meaningful values for P are indicated by Us. The horizontal broken line at $R=2.5 \times 10^7$ indicates our estimate of R for whole mantle convection. The model solutions were obtained on finite-difference grids with dimensions of 24×24 for $R \leq 6 \times 10^5$, 48×48 for $R=1.6 \times 10^6$, 64×64 for $R=5 \times 10^6$ and 96×96 for $R \geq 1.25 \times 10^7$.

The joint dependence of P on both μ and R is summarized in Fig. 3. Here P is plotted as a function of μ and R . Values of P are indicated for the states examined and manually drawn contours for constant P indicate the trends. For a given amount of flattening, the value of μ required is a decreasing function of R .

If convection involving the ocean floor is confined to the upper 700 km of the mantle^{2,5,6,10} the aspect ratio of the flow is much greater than 1 and the results presented here (for an aspect ratio of 1) cannot be applied directly. The most direct application to the Earth is in the context of whole mantle convection, as originally envisaged by Hess¹³, in which it is assumed that the oceanic lithosphere forms the thermal boundary layer of the convective circulation¹⁵. In this case a cell with an aspect ratio of 1 (width=2,900 km at mid-depth) would have a linear surface dimension of 3,752 km, comparable to the mean plate size. Allowing for the geometrical effects of curvature in the spherical Earth we estimate a value for the heat flux E at mid-mantle depths of $9.34 \times 10^{-2} \text{ W m}^{-2}$. Substituting this value for E into equation (2), together with the following parameter values

$$g = 10^3 \text{ cm s}^{-2}$$

$$\alpha = 1.4 \times 10^{-5} \text{ K}^{-1}$$

$$\rho_m = 4.9 \text{ g cm}^{-3}$$

$$d = 2.9 \times 10^3 \text{ km}$$

$$\eta = 5 \times 10^{22} \text{ P}$$

$$\kappa = 2.5 \times 10^{-2} \text{ cm}^2 \text{ s}^{-1}$$

$$C_p = 1.2 \text{ J g}^{-1} \text{ K}^{-1}$$

(assumed to be representative of the whole mantle), gives an estimated value of $R=2.5 \times 10^7$ for mantle-wide circulation. The extrapolated contours on Fig. 3 suggest that, at $R=2.5 \times 10^7$, 10–20% flattening can be expected provided $0.1 \leq \mu \leq 0.2$; the data from the ocean floor¹ indicate a value of $P=18\%$. Thus in the context of the present constant property model, if radiogenic heat sources are uniformly distributed throughout the mantle, they must contribute less than 20% to the total heat flow at the Earth's surface. (A larger contribution would result in flattening in excess of the observations.) Consequently, the large scale convective circulation must be driven substantially by the flow of heat across the core–mantle boundary.

Other processes not included in the simple convection models studied here (notably the rigid behaviour of the lithosphere) undoubtedly will alter, quantitatively, the results presented here. In particular the amount of internal heating compatible with the observations may well exceed 20%. Moreover, application to the Earth is complicated by the presence of continents on many of the large plates. The main result to which we wish to draw attention here is that the topography deduced from the thermal structure at the upper surface of a convection cell is significantly flattened by partial internal heating.

Received 4 February; accepted 11 April 1980.

1. Parsons, B. & Sclater, J. G. *J. geophys. Res.* **82**, 803–827 (1977).
2. Parsons, B. & McKenzie, D. P. *J. geophys. Res.* **83**, 4485–4496 (1978).
3. McKenzie, D. P. *J. geophys. Res.* **72**, 6261–6273 (1967).
4. Sclater, J. G. & Francheteau, J. *Geophys. J. R. astr. Soc.* **20**, 509–542 (1970).
5. Turcotte, D. L. & Oxburgh, E. R. *J. Fluid Mech.* **28**, 29–42 (1967).
6. Turcotte, D. L. & Oxburgh, E. R. *Ann. Rev. Fluid Mech.* **4**, 33–68 (1972).
7. Robinson, J. L. *J. Fluid Mech.* **30**, 577–600 (1967).
8. Roberts, G. O. *Geophys. Astrophys. Fluid Dynamics* **12**, 235–272 (1979).
9. Olson, P. & Corcos, G. M. *Geophys. J. R. astr. Soc.* (in the press).
10. McKenzie, D. P., Roberts, J. M. & Weiss, N. O. *J. Fluid Mech.* **62**, 465–538 (1974).
11. Jarvis, G. T. & McKenzie, D. P. *J. Fluid Mech.* **96**, 515–583 (1980).
12. Oxburgh, E. R. & Turcotte, D. L. *Rep. Prog. Phys.* **41**, 1249–1312 (1978).
13. Hess, H. H. in *Petrological Studies: A Volume in Honor of A. F. Buddington* (eds Engel, A. E. J., James, H. L. & Leonard, B. F.), 599–620 (Geological Society of America, 1962).
14. McKenzie, D. P. *J. R. astr. Soc.* **48**, 211–238 (1977).
15. Peltier, W. R. in *Physics of the Earth's Interior* (eds Dziewonski, A. & Boschi, E.) (Academic, New York, 1980).

Asymmetric spreading in back-arc basins

Peter F. Barker

Department of Geological Sciences, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK

Ian A. Hill

Department of Geology, University of Leicester, University Road, Leicester LE1 7RH, UK

Lineated magnetic anomalies indistinguishable from those generated at mid-oceanic ridges have been found behind many subduction zones. They show that back-arc spreading, like its mid-oceanic counterpart, is to a first approximation symmetric. Nevertheless, consistently asymmetric spreading has been found in some ocean basins, and in both actively spreading and extinct back-arc basins. We argue here that the sense of asymmetry found in active back-arc basins strongly supports a mechanism for the origin of asymmetric spreading which is dominated by the thermal effects of migration of the ridge crest over the sub-aesthenospheric mantle. Conversely, it provides no support for the simple fluid mechanical models, nor for models in which back-arc extension is driven directly by the subduction process. If the thermal effect also dominated asymmetric spreading in extinct back-arc basins, the sense of asymmetry would provide information about 'absolute' plate motions at the time of opening.

Divergent plate boundaries accrete asymmetrically by the action of ridge jumps, or by a kind of smoothly asymmetric spreading in which the discontinuities, if any, are too small to be detected using magnetic anomalies¹⁻⁴. Although the two modes can co-exist their origin is probably essentially different. Unlike all but the smallest ridge jumps, asymmetric spreading is a ridge crest phenomenon, produced in the central rift zone in conditions perhaps only marginally different from those giving rise to the more usual symmetric spreading. We consider here only the smooth asymmetry, despite obvious resemblances between the larger ridge jumps in the main ocean basins and episodic back-arc extension, in which (as in the Philippine Sea^{5,6}) an old centre is abandoned in favour of new spreading directly behind the magmatic arc.

Certain detection of smoothly asymmetric spreading requires detailed examination of long and well-formed magnetic anomaly sequences, to rule out the spurious effects of undetected ridge jumps, fracture zones and changes in ridge obliquity. Hence the validity of some, particularly older, claims of asymmetric spreading in the main ocean basins must be debatable. Reasonably well-documented examples, however, often with one sense of asymmetry persisting over several million years, are found south of Australia^{1,2}, in the South Atlantic³ and Panama Basin⁷⁻¹⁰ and, inferentially¹¹, on the East Pacific Rise.

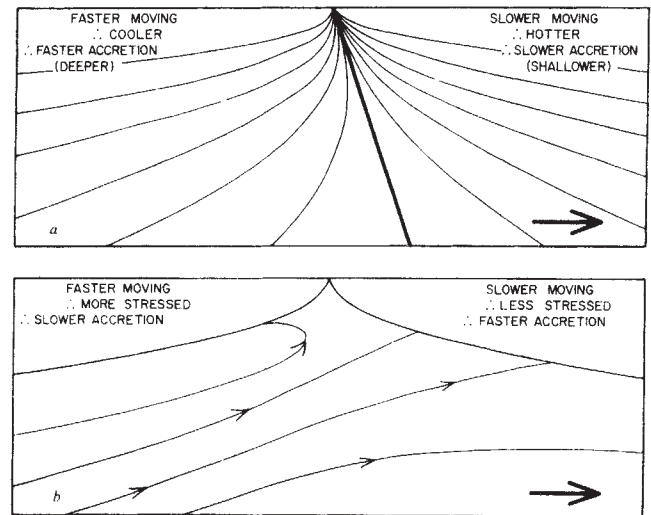


Fig. 1 Simple schematic models relating asymmetric spreading to ridge crest migration over the underlying mantle. Both cases are shown in a ridge crest reference frame with mantle moving to the right (thick arrow). Thus, with respect to the mantle, the left flank of the spreading centre is actually the faster moving. *a*, Thermal model^{1,3}. Migration is represented by sloping the central injection boundary (thick line) of the conventional numerical models of oceanic lithosphere formation¹², which skews the isotherms so that the slower moving (right) flank is hotter for any given depth and age and, therefore, accretes less and rides higher. This elevation asymmetry should decay with age of the ocean floor. *b*, Fluid mechanical model¹³, in which the greater viscous shear stress beneath the faster moving (left) flank hinders accretion. Solid arrowed lines are schematic contours of stream function.

Smoothly asymmetric spreading has been associated with migration of the ridge crest across the underlying mantle in two models (Fig. 1), one invoking the thermal asymmetry produced by ridge migration^{1,3}, the other¹³ the asymmetric shear stresses beneath the diverging plates. The models predict asymmetric accretion in opposite senses; accretion favours the cooler flank, which is the faster-moving, but also the less stressed flank, which is the slower moving.

Using recent versions^{14,15} of the mean hotspot reference frame (MHRF) to compute absolute motions, for the better documented examples (south of Australia, the South Atlantic and, with much greater complexity, the Panama Basin) the sense of asymmetry suggests the dominance of the thermal effect. However, the entire data set from the main ocean basins does not provide overwhelming support to either model and it is not clear why, when almost all mid-oceanic ridges are migrating to some extent¹⁶, asymmetric spreading is not much more widespread. An additional enabling or decoupling mechanism may exist, perhaps within processes in the upper (magma chamber, sheeted dyke) levels of new oceanic lithosphere^{1,3} where the mode of operation of either model in the simplistic form described here is in any case unclear.

The existence of asymmetric spreading in back-arc basins provides the opportunity to examine in a different environment the models discussed so far, and to compare their predictions with those of the subduction-specific class of model for back-arc extension, notably the 'forced convection' model of Toksoz and Bird¹⁷. To test the ridge migration models, the back-arc basins must be actively extending so that absolute motions may be derived, and must possess well-formed, unambiguously identified Vine-Matthews magnetic lineations. Only the South Sandwich¹⁸ and Bismarck Sea¹⁹ basins meet these criteria at present. The Lau²⁰ and Marianas²¹ basins are also active, and show lineated magnetic anomalies, but present data are inadequate for unambiguous identification of the anomalies and the location and orientation of fracture zones. Both the South

Table 1 Absolute plate motion vectors in areas with asymmetric spreading

Area	Position	Plate	Motion (mm yr ⁻¹)	Vector ¹⁵ (°)	Motion (mm yr ⁻¹)	Vector ¹⁴ (°)
Australia–Antarctic Ridge	50°S 130°E	IND	76	009	65	015
South Atlantic	49°S 15°W	ANT	6	041	13	132
		SAM	21	258	20	292
		AFR	14	083	22	052
Panama Basin	2°N 90°W	NAZ	47	086	54	084
		COC	84	041	90	041
Sandwich Plate	60°S 30°W	SAM	17	256	16	302
		ANT	5	182	9	026
Bismarck Sea	4°S 150°E	IND	72	019	66	031
		PAC	101	294	91	288
Shikoku Basin	30°N 137°E	EUR	3	055	12	151
		PAC	107	288	97	282

Sandwich and the Bismarck Sea basins show asymmetric spreading, the sense of which is compatible with the dominance of a thermal effect. In the South Sandwich system (Fig. 2a and Table 1) the trench and arc (Sandwich plate) migrate rapidly eastwards in absolute terms ($60\text{--}80\text{ mm yr}^{-1}$), while the remnant arc moves only slowly westwards ($0\text{--}20\text{ mm yr}^{-1}$). (The range of speeds results from the unknown apportionment of the slow east-west SAM-ANT motion between the North and South Scotia Ridge²²). The ridge crest migrates eastwards at $15\text{--}35\text{ mm yr}^{-1}$ and the asymmetry, $\sim 10\%$ of the total spreading rate, favours accretion to the eastern, trench flank, in agreement with the prediction of a thermal model.

The back-arc spreading centre in the Bismarck Sea¹⁹ is shown in Fig. 2b as part of the Pacific-Bismarck plate boundary. The Pacific (PAC) plate is here moving rapidly to the west-northwest (Table 1), but even more rapid back-arc extension ($\sim 130\text{ mm yr}^{-1}$) permits the New Britain arc-trench system to advance to the east-southeast. The ridge crest migrates slowly to the west-northwest, so that the observed 10% asymmetry, which here favours the remnant arc (western, PAC) flank, is again compatible with a thermal model. The existence of a Caroline plate, with the slow movement relative to the Pacific plate suggested by Weissel and Anderson²³, would not affect this conclusion.

The value of these two examples extends beyond their support for a dominantly thermal model. The sense of the asymmetry, although consistent with respect to the 'absolute' migration of the ridge crest, is not consistent in terms of the polarity of the arc and trench. That is, in one case the asymmetry favours accretion to the trench side, in the other to the remnant arc. We regard this as strong evidence against those models, such as the 'forced convection' model¹⁷, which drive back-arc extension directly from the subduction process, and for which (whether generated thermally or by stress) a consistent sense of asymmetry with respect to the trench would be expected. Rather, these results emphasize the sensitivity of back-arc extension to absolute plate motions, and to this extent support the suggestion by Chase²⁴, that back-arc extension is induced so that the trench may 'advance' over the sub-asthenospheric mantle. Note that the recently reported Bismarck Sea spreading also supports Chase's hypothesis more directly; without it, the trench would be 'retreating' extremely rapidly.

Unfortunately, no other active back-arc basins with well-formed, unambiguously identified magnetic anomalies are known at present: the two cited provide more emphatic support for a thermal model than do the main ocean basins. If further

support were to become available from other examples, it would be reasonable to assume that the association of asymmetric spreading with ridge crest migration could be applied also to extinct back-arc basins. Provided that the regional tectonic situation was fairly simple while extension was taking place, then the observed sense of asymmetry might be used to deduce the polarity of ridge crest migration in the direction perpendicular to the crest itself. We choose two examples, the central Scotia Sea and the Shikoku basin, where such information would contribute towards an understanding of regional tectonic evolution.

East-west magnetic lineations²⁵ in the central Scotia Sea (Fig. 2c) reveal a spreading episode extending from 21 to 6 Myr, with an asymmetry of $5\text{--}10\%$ favouring accretion to the southern flank. During this period, major plate (SAM-ANT) motion was probably slow, sinistral and east-west, as it is today^{14,15}. South Sandwich back-arc extension¹⁸ had not yet begun, but extension in the $120\text{--}300^\circ$ direction was taking place in Drake Passage to the west²⁶. Recently dredged Miocene low-K arc tholeiites from the eastern South Scotia Ridge²⁷ suggest that the central Scotia Sea formed by back-arc extension behind a southeast-facing intra-oceanic arc, the Discovery Arc, as shown in Fig. 2c. However, the nature of the coupling between the various spreading systems is uncertain, so that, although about two-thirds of the Scotia Sea floor is now dated, its tectonic evolution is not yet fully understood. In particular, subduction at the Falkland trough, beneath a northward-migrating North Scotia Ridge²⁸ (shown unshaded in Fig. 2c) may conceivably have been responsible for central Scotia Sea opening, in different coupling conditions. The sense of the asymmetric spreading, if compatible with a thermal model, would suggest that the ridge crest was migrating to the south, and therefore that most if not all of the subduction was taking place at the Discovery Arc, as shown in Fig. 2c.

The Shikoku Basin lies at present on the Philippine plate, which is itself being subducted slowly in the north-west beneath the Eurasian plate, while its eastern margin is the site of fast subduction of Pacific plate. A recent re-evaluation of all available Shikoku Basin magnetic data by Shih²⁹ shows that the basin opened between ~ 24 and 15 Myr ago, with an asymmetry of $5\text{--}7\%$ favouring accretion to the western, remnant arc side. The thermal model predicts from this a component of ridge crest migration along 250° , and therefore a migration of the western flank of the ridge crest in the same direction at a rate at least as fast as the half spreading rate ($17\text{--}24\text{ mm yr}^{-1}$). If the absolute motions of the Pacific and Eurasian plates were then similar to those of today (Fig. 2d and Table 1), this migration alone would require subduction of the western flank beneath the Eurasian plate, independently of any northward movement implied by palaeomagnetic evidence⁵.

This raises another point of interest. The direction of back-arc basin opening within the Cenozoic, with respect to the island arcs and also probably in absolute terms, has been largely east-west, a motion not amenable to detection by palaeomagnetic means. If a consistent ridge migration/asymmetry relationship were to be established, it would be possible to detect at least the polarity of such palaeomotion. If, in addition, Chase's subduction imperative²⁴ that trenches advance were accepted, the motions of some island arcs could be deduced within quite narrow limits. At the time of Shikoku Basin opening, for example, the palaeo-Mariana/Bonin arc would have been advancing eastward more slowly than the half-spreading rate (15 to 22 mm yr^{-1}). Such data would be of value in studies of the global distribution of plate motions at times in the past, aimed at modelling the driving forces³⁰, but it should be remembered that the MHRF, with all its assumptions, would have been involved in their production.

As in the active back-arc examples, the sense of asymmetric spreading in one of the extinct basins described above favours accretion to the remnant arc side, and in the other to the side carrying the island arc and trench; this adds to the argument against 'forced convection' models of back-arc extension. Thus, although some of the foregoing discussion is undoubtedly pre-

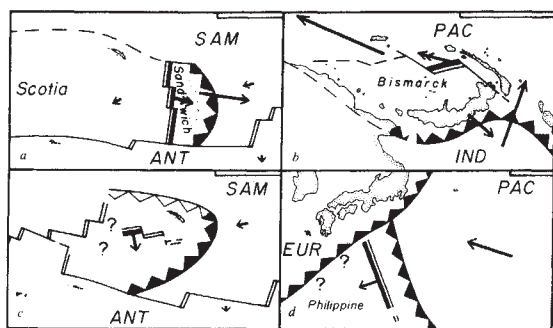


Fig. 2 Asymmetric spreading in presently active East Scotia Sea¹⁸ (a) and Bismarck Sea¹⁹ (b), and extinct central Scotia Sea²⁵ (c) and Shikoku⁵ (d) back-arc basins. Spreading ridges are double lines, the thicker indicating the faster-spreading flank. Single-headed thick arrows are 'absolute' plate motions, from ref. 15 (Table 1) or as derived in the text. Double-headed arrow (a and b) is absolute motion of ridge crest. Length of line in upper right of each diagram shows 500 km distance and 100 mm yr^{-1} arrow length. Extinct back-arc basins (c and d) both shown as at ~ 15 Myr ago, but with present-day major plate motions; spreading centre arrows show ridge crest migration component compatible with thermal effect.

mature, being based on so few examples, it is clear that asymmetric spreading is of some interest both as a means of understanding active margin processes and for its possible use in estimating components of absolute plate motions in the past.

Received 18 January; accepted 2 May 1980.

1. Hayes, D. E. *Bull. geol. Soc. Am.* **87**, 994–1002 (1976).
2. Weissel, J. K. & Hayes, D. E. in *Antarctic Research Series* Vol. 19, 165–196 (American Geophysical Union, Washington, DC, 1972).
3. Barker, P. F. *Geophys. J. R. astr. Soc.* **59**, 131–145 (1979).
4. Macdonald, K. C. *Bull. geol. Soc. Am.* **88**, 541–555 (1977).
5. Karig, D. E. in *Init. Rep. DSDP Leg 31*, 857–879 (1975).
6. Watts, A. B., Weissel, J. K. & Larson, R. L. *Tectonophysics* **37**, 167–181 (1977).
7. Hey, R., Johnson, G. L. & Lowrie, A. *Bull. geol. Soc. Am.* **88**, 1385–1403 (1977).
8. Hey, R. *Bull. geol. Soc. Am.* **88**, 1404–1420 (1977).
9. Lonsdale, P. & Klitgord, K. D. *Bull. geol. Soc. Am.* **89**, 981–999 (1978).
10. Hey, R. *Geology* **7**, 504–506 (1979).
11. Elvers, D., Srivastava, S. P., Potter, K., Morley, J. & Seidel, D. *Earth planet. Sci. Lett.* **20**, 211–219 (1973).
12. Selater, J. G. & Francheteau, J. *Geophys. J. R. astr. Soc.* **20**, 509–542 (1970).
13. Stein, S., Melosh, H. J. & Minster, J. B. *Earth planet. Sci. Lett.* **36**, 51–62 (1977).
14. Chase, C. G. *Earth planet. Sci. Lett.* **37**, 355–368 (1978).
15. Minster, J. B. & Jordan, T. H. *J. geophys. Res.* **83**, 5351–5354 (1978).
16. Solomon, S. C., Sleep, N. H. & Richardson, R. M. *Geophys. J. R. astr. Soc.* **42**, 769–801 (1975).
17. Toksoz, M. N. & Bird, P. in *Maurice Ewing Series* Vol. 1, 379–393 (American Geophysical Union, Washington DC, 1977).
18. Barker, P. F. *Earth planet. Sci. Lett.* **15**, 123–132 (1972).
19. Taylor, B. *Geology*, **7**, 171–174 (1979).
20. Weissel, J. K. in *Maurice Ewing Series* Vol. 1, 429–436 (American Geophysical Union, Washington DC, 1977).
21. Karig, D. E., Anderson, R. N. & Bibee, L. D. *J. geophys. Res.* **83**, 1213–1226 (1978).
22. Forsyth, D. W. *J. geophys. Res.* **80**, 1429–1443 (1975).
23. Weissel, J. K. & Anderson, R. N. *Earth planet. Sci. Lett.* **41**, 143–158 (1978).
24. Chase, C. G. *J. geophys. Res.* **83**, 5385–5387 (1978).
25. Hill, I. A. & Barker, P. F. *Geophys. J. R. astr. Soc.* (in the press).
26. Barker, P. F. & Burrell, J. *Mar. Geol.* **25**, 15–34 (1977).
27. Barker, P. F., Hill, I. A., Weaver, S. D. & Pankhurst, R. J. in *Antarctic Geoscience* (University of Wisconsin Press, Madison, in the press).
28. Ludwig, W. J., Windisch, C. C., Houtz, R. E. & Ewing, J. I. in *Am. Ass. petrol. Geol. Mem.* **29**, 125–137 (1979).
29. Shih, T. *Int. Rep. DSDP* (in the press).
30. Solomon, S. C., Sleep, N. H. & Jurdy, D. M. *J. geophys. Res.* **82**, 203–212 (1977).

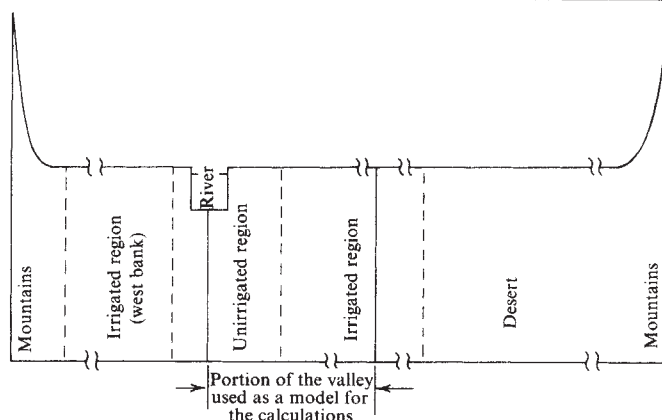


Fig. 1 Cross-sectional diagram of Nile Valley in Karnak Temple area (not drawn to scale).

time of the Middle Kingdom until Ptolemaean time, and consists of smaller temples, shrines, courts, halls and chapels. It fell into disrepair in Roman times, ~2,000 yr ago.

The physical system considered in this simulation is shown in Fig. 1 and is represented by a river valley between two rows of mountains impermeable to water. A large area of the valley is irrigated but between this area and the river is an unirrigated region where the temples are situated. Mathematically, this is a two-dimensional system, because the system is approximately uniform in the y direction which is parallel to the river. The dimensions are defined by the direction, x , normal to the river towards the irrigated land, and the direction, z , normal to the ground surface, that is, the depth. We considered a portion of the physical system, as shown in Fig. 1 for our calculations. As we are interested in the average processes occurring in the valley, and because the climate and hydrology of the valley are relatively stable¹, the system was assumed to be in a steady state.

The difference in the levels of the Nile and the water table under the irrigated fields constitutes a driving force that moves the water and soluble salts from the fields into the river. As there is no irrigation in the temple area, the evaporation of moisture at the surface of the ground constitutes another driving force that moves saline water from the water table to the surface, where salts, which had been in solution, are deposited. The relative importance and the approximate rates of these processes were determined here for the water saturated region by solving the steady-state continuity equation, with Darcy's law

$$J = K \nabla P$$

defining the flux, where K is the hydraulic conductivity, and P the gravitational potential plus hydrostatic pressure potential.

The steady-state continuity equation is

$$\nabla J = K \nabla^2 P = 0$$

therefore

$$\nabla^2 P = 0$$

The solution to this equation for the Karnak area is most easily done numerically due to the nonlinearity of the boundaries of the system.

Initially the IBM 370-65 and 3300 computers were used for these calculations; however, the final work was done using a SEL 55 mini-computer which was approximately 16 times slower than the IBM 3300. The computer program developed yielded a solution for the equation which was stable, consistent and convergent, and required typically 5 h of the mini-computer time. The effort in this work was in obtaining

Solution of the continuity equation for the Karnak area

T. C. Billard & George Burns

Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Concern has long been expressed that ancient Egyptian temples alongside the Nile deteriorate with time. Deteriorations were usually associated with annual inundations. When flood waters receded, salt deposits attacked the porous sandstone, reducing it to sand, and thus destroying the foundations. With the construction of the first dam, and of the High Dam near Aswan (in 1965), the annual inundations were eliminated. However, monitoring of the deterioration continued, especially in the Thebes-Karnak area¹, where the temples are particularly numerous and important. The dam's construction was generally considered beneficial² mostly because earlier causes of land salinization were eliminated. But this consensus was based on hydrological, climatological and chemical data¹ and was only qualitative, and it has not been possible to answer this question even semi-quantitatively. However, visual observation indicates that some form of deterioration, due to salts appearing at the foundation of some monuments in Egypt, still takes place. Consequently, a deeper understanding of salinization processes is needed. We describe here a computer simulation of the flow of saline water in the area of the Great Temple of Karnak, to determine whether salinization in the post-dam, that is, present time, is a serious threat to the antiquities there.

The Karnak Temple is situated on the east bank of the Nile ~450 m from the river. It is ~500 m wide, and together with adjacent temple complexes, stretches along the Nile for about 1 km. It was constructed over a period of ~2,000 yr from the

significant data, rather than in developing an efficient computer program. Therefore, further improvement in computer time used may be anticipated.

The hydraulic conductivity of the soil was chosen to be $0.003 \text{ cm min}^{-1}$. This value is in the low range of alluvial soil hydraulic conductivities, and it was selected to obtain a conservative estimate of water flow. The flow results, including the amount of salts deposited in unirrigated areas, are directly proportional to the hydraulic conductivity. Although the soil of the system is not uniform, for calculating average water flow in and out of the system, it is reasonable to assume soil uniformity (constant K).

Figure 2 shows the movement of the underground water in the Karnak area. These results were obtained from solutions of the continuity equation with boundary conditions deduced from the geological survey map of the Luxor-Karnak area, from geological surveys^{3,5} and from previously published data¹. The boundary conditions are: (1) no flow, $(\partial P/\partial x)=0$, at the water divide in the irrigated fields, and at the bedrock, $(\partial P/\partial z)=0$, which is the lower boundary; (2) no flow below the river from one bank to the other, $(\partial P/\partial x)=0$; (3) zero-potential, $(P=0)$, at the river/soil boundary; (4) the potential at the upper boundary is equal to the height of the water table, above the river height.

The key boundary condition is the height of the water table. At Karnak, it is an experimentally known quantity¹ and implicitly includes the effect of the evaporation at the ground surface, because the position of the water table is determined by the extent of irrigation and evaporation.

The flows of water across the boundaries were calculated and are given in Table 1. These results for the underground water flow for the Karnak regions show that $\sim 92\%$ of the water, which drains from the irrigated land, is evaporated on or near the surface of the unirrigated land, including the Karnak Temple site. The concentration of salts in the underground water in the Karnak area was determined to be on average $15 \text{ m Equiv. l}^{-1}$ (ref. 1) which is in good agreement with our own measurements. This yields the average rate of salt deposition in the immediate area of the Karnak Temple area as $0.04 \text{ Equiv. yr}^{-1} \text{ m}^{-2}$. As the soil conductivity was chosen conservatively, the average salt deposition calculated above is also a conservative number.

Although pre-Aswan Dam deterioration of the Karnak Temples has been eliminated, we conclude that new physicochemical processes operate since the Aswan Dam was built, causing salinization of land and ancient monuments.

Salinization occurs because irrigation raises the water table and causes a flow of saline underground water, which evaporates over dry unirrigated land. As archaeological monuments in the Nile Valley were commonly situated on dry

Table 1 Flows in the water saturated system per metre parallel to the river

Flow into the system through the irrigated fields ($\text{m}^2 \text{ min}^{-1}$)	6.1×10^{-5}
Flow out of the system into the river ($\text{m}^2 \text{ min}^{-1}$)	0.3×10^{-5}
Flow out of the system through the water table ($\text{m}^2 \text{ min}^{-1}$)	5.8×10^{-5}

land in the vicinity of irrigated fields, one would expect that processes of salinization, as described in the present model, occur not only in the Karnak area, but also elsewhere in Egypt. Such salinization endangers the foundations of the temples and unexcavated antiquities. As these processes are continuous and cumulative, over a long period of time, they threaten the very existence of the great monuments.

These calculations are being refined and the method will be applied to other regions, where hydrological data¹ are available.

We thank Dr N. W. Reid for his interest, the Egyptian Antiquities Organization for a permit to commence work, and particularly Drs Ali el Khouli, Zaki Iskander and Abdel Kadar Selim. Continuation of this work was made possible through the encouragement of Dr Shahata Adam, President of the Egyptian Antiquity Organization. Our field work was greatly helped by Mr Mohammed el Saghir and Mr el Sayd Abel el Hamid. This work was supported in part by the Natural Sciences and Engineering Council, and in part by the Social Sciences and Humanities Research Council of Canada.

Received 31 December 1979; accepted 17 April 1980.

1. Traunecker, C. *Kémi* 20, 195 (1970); 20, 213 (1970); 21, 177 (1971); 21, 197 (1971); *Karnak* 5, 119 (1975); 5, 131 (1975).
2. Lauffray, J. *Le Courrier du CNRS* 9, 28 (1973).
3. Attia, M. L. *Geological Survey of Egypt-Deposits in the Nile Valley and the Delta* (Government Press, Cairo, 1954).
4. Hume, W. F. *Survey of Egypt-Geology of Egypt* (Government Press, Cairo, 1925).
5. Lyons, H. G. *The Physiography of the Nile River and its Basins* (Finance Department of Egyptian Government, Cairo, 1906).
6. *Conservation Problems in Egypt* (UNESCO Consultant Report, Contract No. 33.591, 1970).

Possible role of abscisic acid in reducing seed set in water-stressed wheat plants

J. M. Morgan

Agricultural Research Centre, RMB 944, Tamworth 2340, Australia

One of the primary effects of water stress on grain yield in wheat is the reduction of the number of seeds per spikelet through an increase in male sterility^{1,2}. However, little is known of the relationship between loss of pollen viability and the water relations of the developing spikelets, so that it is not known whether the effect of water deficit occurs as direct dehydration of the anther or by some other means. We report here that seed set may be reduced by water deficits which cause leaf wilting but have no effect on spikelet turgor or relative water content. This raises the possibility that abscisic acid (ABA), which is known to increase substantially when the leaf wilts^{3,4} and to modify developmental processes in plants⁵, is involved in the processes causing floret infertility. In support of this hypothesis we show that reductions in seed set due to water stress are accompanied by increases in endogenous ABA in both leaf and spikelet, and that foliar application of synthetic ABA at the stage of meiosis also reduces seed set and causes malformation of pollen grains and anthers.

The relationship between leaf turgor and seed set was derived from studies on wheat plants (*Triticum aestivum* L. cultivar Condor) which were grown in pots containing equal volumes of sand and peat moss in a glasshouse. The plants

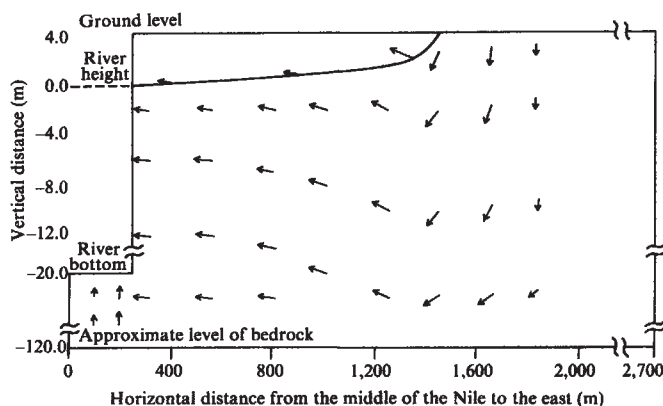


Fig. 2 Water flux on the east bank of the Nile River at Karnak. The water table level is indicated by a curve which extrapolates into the river height. (Relative magnitude of flux is on a log_e scale.)

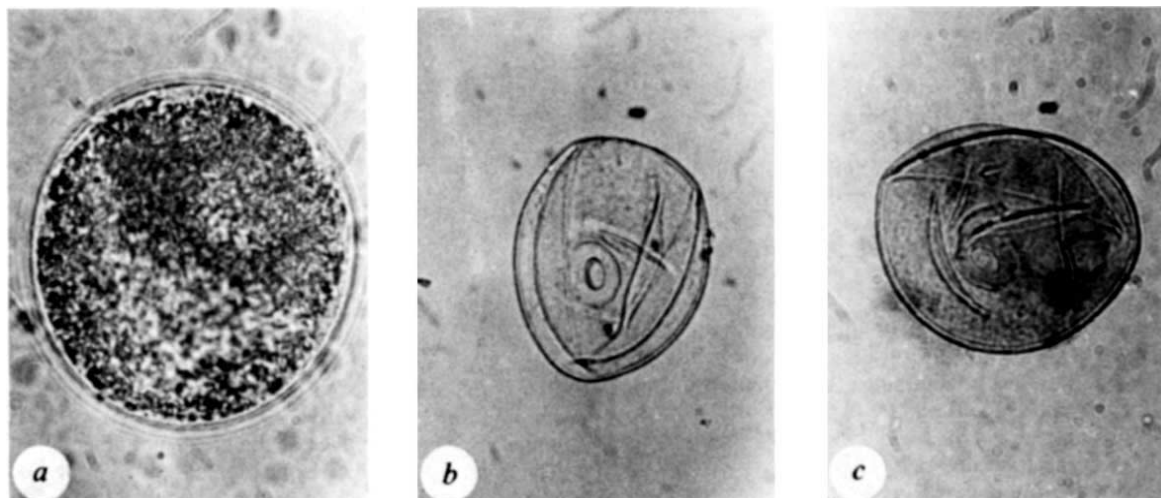


Fig. 1 Mature pollen grains from well watered (a), water stressed (b) and ABA-treated (c) plants ($\times 1,250$).

were watered daily and received standard nutrient solution twice weekly in sufficient quantity to saturate the soil and provide a small amount of drainage. Water stress was produced by withholding water when the anthers of basal florets in the centre of the ears of the primary tillers were near to the stage of meiosis. This stage was reached when the flag leaf ligule was ~ 4 cm above the ligule of the enclosing leaf. Pilot experiments and published results² have indicated that male sterility results from water stress at this stage. Tillers which were 2–4 d more advanced (early booting) were also sampled. Measurements of water (ψ), and osmotic (π) potentials (using Spanner-type thermocouple psychrometers⁶) and relative water contents (ζ) (using the technique of Slatyer and Barrs⁷) were made on tillers with leaves which had just wilted or were at higher water potentials; samples were also taken from unstressed plants. (Turgor potential, P , was taken as $\psi - \pi$). The first signs of wilting occurred within about 6 d of withholding water. Each sampled tiller was matched with a labelled tiller at the same stage of development and the pots were rewatered. After maturity, seeds and florets were counted on ears of tagged tillers.

Table 1 Seed set and water relations of spikelets of plants with wilted and turgid leaves

Stage of development when stress applied	Range of leaf turgor (MPa)	Spikelet		
		Mean P (MPa)	Mean ζ	% fertile florets
Meiosis	0.1–1.2	0.6 ± 0.1	0.93 ± 0.06	67.8 ± 1.5
	–0.1–0.1	0.6 ± 0.1	0.95 ± 0.01	44.0 ± 2.6
Early booting	0.2–1.0	0.6 ± 0.1	0.94 ± 0.04	67.8 ± 1.9
	–0.1–0.0	0.6 ± 0.1	0.94 ± 0.02	43.0 ± 2.0

The most significant result is that there was no change in seed set until the leaf had reached approximately zero turgor (Table 1). Thus, at meiosis, the data could be formed into two groups, depending on whether seed set had been reduced or not; one covering the range of P from 0.1 to 1.2 MPa in which there was no change in seed set and the other for the range of P from –0.1 to 0.1 MPa in which there were 36% fewer seeds per floret. Tillers at the early booting stage showed the same response. This reduction in seed set was not related to either spikelet turgor or spikelet relative water content. There was no difference in either of these parameters between plants with

and without reduced seed set (Table 1) because reductions in spikelet water potentials were fully matched by reductions in osmotic potential. Because the onset of water stress was rapid, the leaves did not show osmotic adjustment, and the turgor declined to zero. Observations of pollen morphology and fertilization tests confirmed that affected florets were male sterile.

The reduction in seed set in the absence of water deficits in the spikelets suggests that the effect is indirect, possibly involving plant growth regulators. Indeed, the coincidence of reduced seed set with loss of leaf turgor suggests the involvement of ABA because the amount of this substance is known to increase substantially when the leaf wilts^{3,4}.

This was confirmed in a second experiment, similar to the first except that levels of ABA were measured in the spikelets and leaves. Endogenous ABA was determined by gas chromatography (electron capture detector) after purification of the samples by solvent partitioning as described by King⁸, except that purification of the methylated samples was achieved using a high pressure liquid chromatograph instead of a thin-layer chromatograph. Recovery was greater than 60%. The concentration of ABA in wilted leaves ($P=0.0$ MPa) was 143 ng g^{-1} fresh weight compared with 23.5 ng g^{-1} in turgid leaves ($P=0.6$ MPa). Furthermore, the levels of ABA in the spikelets of plants with wilted and turgid leaves were 111.0 ng g^{-1} and 35.2 ng g^{-1} , respectively, even though there was no difference in spikelet turgor potential (mean $P=0.6$ MPa). The seed set, however, was reduced from 55% in turgid plants to 4% in wilted plants.

On the basis of these results and published data^{3,4}, it seemed possible that a reduction in seed set due to water stress could be linked with an increase in the level of ABA due to leaf wilt. To test this hypothesis, synthetic ABA was applied to the flag leaves of tillers when the basal florets of spikelets in the centre of the ear were between meiosis and second mitosis, and after second mitosis (boot swell stage). The plants were grown in the same way as described above. Aqueous solutions of ABA of 1, 10 and 30 mg l^{-1} were made up by dissolving the ABA in $\sim 10 \text{ ml}$ of 1 M KOH , diluting with water and adjusting the final solution to pH 5.8. The control solution was the same but contained no ABA. The solutions were applied by immersing the leaves for 2 min. Each treatment was replicated five times. Tillers were tagged and observations were made of pollen morphology just before anthesis by squashing the anther in water or acetocarmine and photographing through a microscope. Several weeks after anthesis numbers of florets and seeds of tagged tillers were recorded.

Applications of ABA of 10 and 30 mg l^{-1} at meiosis reduced seed set by 43 and 27% (significant at 0.1% level) below

control values respectively, but 1 mg ABA l^{-1} had no effect (Table 2). At the later stage there was a reduction of 8% (non significant) when 30 mg ABA l^{-1} was used, but no effect when lower levels of ABA were used. The reductions in seed set due to applications at meiosis were associated with distorted pollen grains devoid of starch granules; these were identical to pollen grains affected by water stress (Fig. 1). Also the anthers, which were reduced in size, pale yellow and often shrivelled, were identical to those affected by water stress. Furthermore, both water stress and treatment with ABA produced a few tillers which failed to develop beyond the early boot swell stage.

Table 2 Effects of ABA on seed set

Stage of development when ABA applied	ABA concentration (mg l^{-1})			
	0	1	10	30
Meiosis	65.6	64.6	37.3	48.2
Booting	61.0	65.1	60.5	56.3

Effects were measured as per cent fertile florets.

From these results it is not possible to determine the precise stage of microsporogenesis at which ABA acts. Although seed set was reduced by applications of ABA when basal florets were between pre-meiosis and second mitosis, other florets, particularly the central ones in the spikelets and those near the tip and base of the ear, would have been at an earlier stage of development. It was usually those that failed to develop grain. It is possible that the effects of ABA are mitigated through the production of ethylene⁹, in which case the ABA could be affecting pollen development in the same way as Ethrel, which is known to break down to produce ethylene in plant cells at low pH. Ethrel causes male sterility only when applied before meiosis¹⁰.

The fact that an increase in the amount of ABA in the leaf through application causes a reduction in seed set and alteration of pollen morphology identical to that caused by water stress supports the hypothesis that water stress affects seed set through an increase in endogenous ABA, though further studies are needed to clarify the mode of action. If this hypothesis is correct, it implies that ABA has a role in regulating grain number in anticipation of further periods of water stress, thereby reducing the number of 'sinks' for carbohydrates, the supply of which would be reduced by stress. The results also highlight the importance of mechanisms which maintain leaf turgor during periods of declining water potential, such as osmotic adjustment¹¹, as a means of reducing yield losses in crop plants growing in semi-arid environments.

The assistance of Mr G. O'Connor, Mr A. Condon, Dr A. Gleeson and Dr R. King and the financial support of the Wheat Industry Research Council of Australia are gratefully acknowledged.

Morphogenesis of branching tubules in cultures of cloned mammary epithelial cells

D. C. Bennett

The Salk Institute, PO Box 85800, San Diego, California 92138

Morphogenesis (the development of biological form, usually of multicellular organisms or their parts) is generally studied in simple organisms like the slime mould *Dictyostelium*¹, for in mammals even single tissues like the mammary epithelium discussed here appear complex. Mammary epithelium, supported by mesenchymal tissue, forms a system of branching, tubular ducts. During phases of rapid growth these ducts end in solid, swollen 'end-buds', and when mature in globular 'alveoli'². The mesenchyme influences the morphogenesis of the epithelium and may be essential for this process^{3,4}. An unknown number of cell types are present in both the epithelium and the mesenchyme. One step towards a better-defined 'model gland' was taken by Yang *et al.*⁵, who recently described three-dimensional, solid, tumour-like outgrowths from clumps of mouse mammary tumour cells cultured in floating 'collagen gel'⁶ instead of on a plastic surface. I now describe behaviour retaining some elements of natural morphogenesis, in a cloned line of epithelial cells and thus in the unequivocal absence of mammary mesenchyme—unless mesenchyme can arise from epithelium. On floating collagen gel Rama 25 cells (derived from a rat mammary tumour⁷) could generate three-dimensional structures which, although often disorganized and tumour-like, included branching, hollow tubules, sometimes with bulbous ends. Thus all the information to specify such organization resided in a single cell type and survived cloning. This raises the possibility of a simple mammalian system in which morphogenetic mechanisms, and their relation to cell differentiation, can be studied as readily as in *Dictyostelium*.

Cultures of Rama 25 'cuboidal' cells were maintained as described previously⁷. These cells behave in culture as 'stem cells': despite repeated cloning they form other types of cells including 'elongated' cells, believed to be mammary myoepithelial cells⁷⁻⁹. Elongated cells show epithelial behaviour during early passages, forming cohesive colonies and resisting detachment by trypsin, but they share some structural and antigenic features with mesenchymal cells^{7,9}. Studies of Rama 25 cells on floating collagen gel were undertaken when Emerman *et al.*¹⁰ reported exceptional functional differentiation in normal, mouse mammary epithelial cells maintained on this substrate. 'Fixed' collagen gels were prepared as described in Fig. 1 legend, or with minor differences in protocol whose effects are analysed below. Rama 25 cells were plated on top. The cells attached and proliferated less quickly than on plastic, but formed a confluent cell layer in 4-9 days. The layer was very dense but predominantly one cell thick⁷. Occasional foci of elongated cells were present beneath the monolayer, but usually no other departures from the monolayer were present at this stage.

The gels were now released to float⁶. Over the subsequent 3-4 weeks, three-dimensional structures of several types appeared, with marked variation between different gels (see below). The most striking type, often observed, consisted of blunt projections into the gel. These were seen from about day 3-7 after floating and were usually in clusters, of which between 5 and 30 generally developed per gel. Branching of these outgrowths was observed (Fig. 1), sometimes up to five times in succession. Sometimes the tips of the branches were bulbous (Fig. 1a), like mammary 'end-buds'². Some such outgrowths resembled early mammary rudiments³. Histological studies showed that these structures were

Received 30 October 1979; accepted 8 April 1980.

1. Salter, P. J. & Goode, J. E. *Crop Responses to Water at Different Stages of Growth* (Commonwealth Agricultural Bureau, Farnham Royal, 1967).
2. Bingham, J. *Ann. appl. Biol.* **57**, 365-377 (1966).
3. Harrison, M. A. & Walton, D. C. *Plant Physiol.* **56**, 250-254 (1975).
4. Zabadal, T. J. *Plant Physiol.* **53**, 125-127 (1974).
5. Quarrie, S. A. & Jones, H. G. *J. exp. Bot.* **28**, 192-203 (1977).
6. Waister, P. D. *Nature* **205**, 922-923 (1965).
7. Slatyer, R. O. & Barrs, H. D. *UNESCO Arid Zone Res.* **25**, 331-342 (1965).
8. King, R. W. *Planta, Berl.* **132**, 43-51 (1976).
9. Goren, R., Altman, A. & Giladi, I. *Plant Physiol.* **63**, 280-282 (1979).
10. Bennett, M. D. & Hughes, G. W. *Nature* **240**, 566-568 (1972).
11. Morgan, J. M. *Nature* **270**, 234-235 (1977).

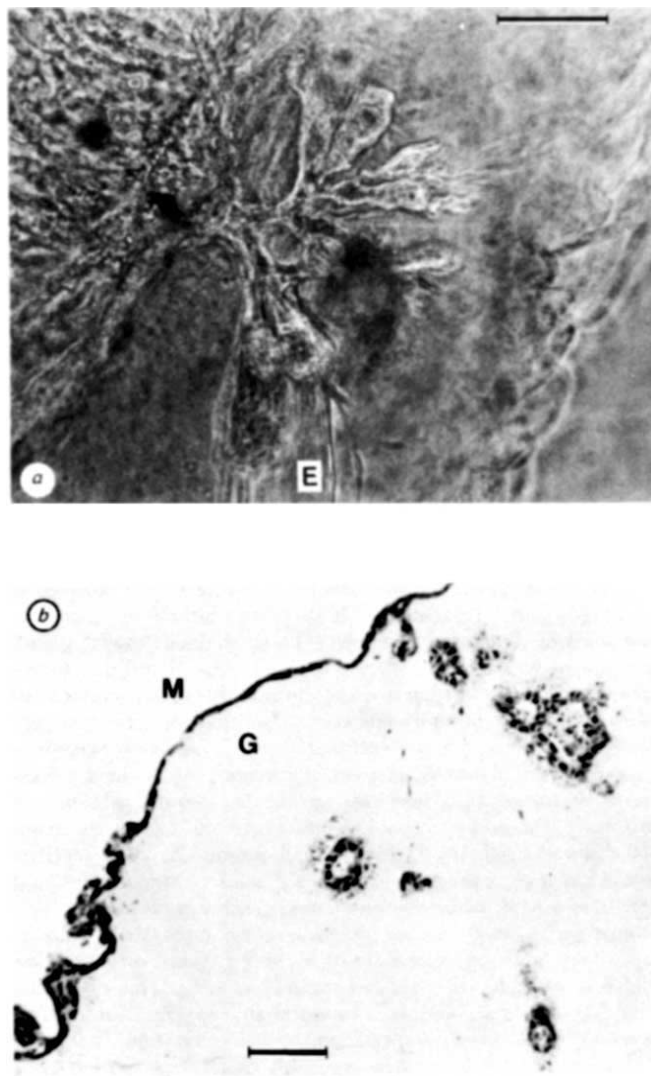


Fig. 1 Gland-like outgrowths from Rama 25 cells on floating collagen gels. Collagen solution was prepared by acetic acid extraction of rat-tail tendons⁶ in sterile conditions then centrifuged for 1 h at 10,000g and the pellet discarded. 1M NaOH solution was mixed with 2× concentrated DEM (Dulbecco's modification of Eagle's medium) to give 0.11 M NaOH, and the precipitate removed using a 0.22 µm filter (Nalge). This medium and the collagen solution were kept at 4 °C. To make each of four gels, 1.5 ml of the alkaline medium were mixed at 4 °C with 5 ml of collagen solution and 1.5 ml were dispensed quickly to each 33-mm culture dish (Falcon). When set, each gel was overlaid with 2 ml of culture medium (DEM with 7% or 10% fetal calf serum), and left for 1 day or more in a humid incubator at 37 °C, gassed with 10% v/v CO₂ in air. A suspension of Rama 25 cells was prepared⁷ and plated on the gels in a fresh 2 ml of the same medium at 2×10^5 cells per dish. Incubation was resumed and the medium was renewed every 2–3 days until the cultures were confluent. Each gel was now released to float⁶ and transferred to an 85-mm, bacteriological grade dish (Falcon) with 10 ml of medium to support the many cells now present. Incubation was resumed, with fresh medium every 5 days or less. *a*, Living culture seen by phase contrast microscopy, 2 weeks after floating, showing outgrowth from cell layer at left, branching about four times and with bulbous ends. *E* denotes chain of elongated cells. *b*, 5 µm paraffin section of similar culture, fixed in neutrally buffered formalin (haematoxylin and eosin stain; transmission optics). *G* denotes gel; *M*, surface of cell sheet which faced culture medium. Several tubules are sectioned, showing branch-points and mostly single-layered epithelium; at left cell layer forms papillae. Scale bars represent 100 µm.

generally hollow tubules (Fig. 1*b*). These tubules resembled tubular carcinoma more closely than normal mammary epithelium: their walls were usually one cell thick (sometimes up to three); the cells were small and somewhat pleomorphic; mitotic figures were seen quite frequently; pyknotic cells were scattered both in and outside some tubules, and the outer layer of myoepithelial cells present in normal mammary ducts² was not observed in these.

Elongated (myoepithelial-like) cells were present focally in all cultures, but they usually migrated into the gel in densely branching chains and spikes instead of forming a second cell layer. Cloned elongated cells of the line Rama 29 (ref. 7) produced similar formations, although these cells also grew preferentially on the gel surface. Mammary cells invading collagen gel have previously been assumed to be mesenchymal^{5,10}. However, these elongated cells arose from cloned epithelial cells and so are probably myoepithelial (see above). Perhaps this behaviour was associated with the failure of Rama 25 cells on collagen gel to produce a continuous basal lamina, as shown by electron microscopy⁷. Some otherwise similar spiky outgrowths had thicker, apparently solid branches and were reminiscent of the predominant structures described by Yang *et al.*⁵. Many small, apparently solid 'lumps' a few cells in diameter appeared from days 1–4. Broader areas with a convoluted surface like that of brain coral arose later, probably by coalescence of the lumps. In section these areas resembled papillary hyperplasia (Fig. 1*b*). Finally, groups of globules or spheres arose from days 16–22, in a few cultures only, either directly from the monolayer or from the tubular outgrowths (Fig. 2). Preliminary histological sections showed, among scattered elongated cells, circular cavities lined by one layer of small, flat cells. Some mitoses were seen, while pyknotic cells were again present. Thus, despite a superficial resemblance to mammary alveoli, these outgrowths were again more like some form of dysplasia.

The variation in the numbers and types of structures produced, between gels and between experiments, seemed largely due to small variations in the preparation of the gel. For example, sterilization of the gels under UV light as generally used^{6,10} proved to cause drying of the gels. When gels were deliberately dried at 37 °C for different times (16–48 h) before use, the longer-dried gels were thin and flaccid when released. Cells initially attached and proliferated very well on these dehydrated gels, but after floating tended to form

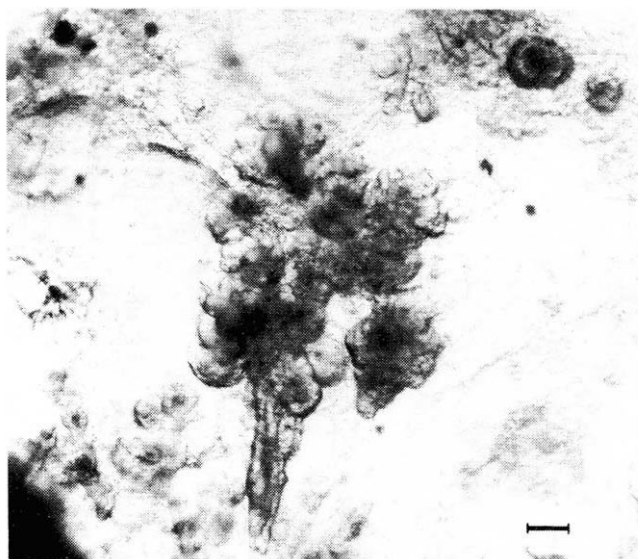


Fig. 2 Later form of outgrowth from Rama 25 cells. The figure shows living culture, prepared as for Fig. 1 and photographed on day 17. Duct-like outgrowths present in this gel up to day 14 had given place to clusters of spheres as shown (only part of cluster in focus). Transmission optics. Scale bar, 100 µm.

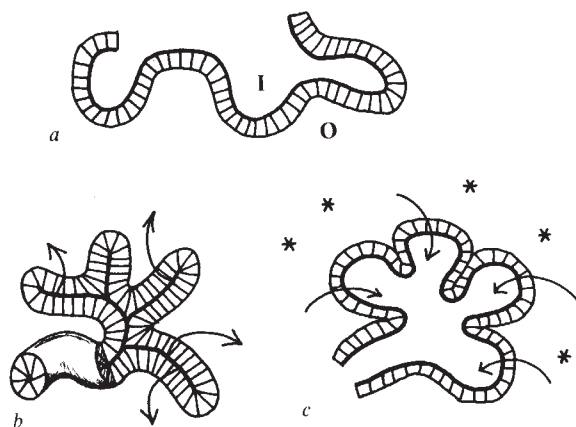


Fig. 3 Hypothetical set of properties allowing cells of a single type to form tubules and alveoli. *a*: (1) Cells cohere to form a membrane. (2) The membrane has limited thickness, for example, a single cell layer with an upper limit to cell height. (3) Cell volume has a lower limit. (4) Cells are readily distorted. (5) The membrane remains continuous (cannot fragment). (6) 'Inside' (I) and 'outside' (O) surfaces are different. *b*: (7) Processes exist which keep the inside surface area at a minimum (zero). The interior is thus one-dimensional: a point for very small membranes or a line, which can branch. The membrane then forms an occluded sphere or (branching) tube respectively. It can grow while maintaining this form. (7a) (optional) if the membrane is relatively impermeable and separates two fluid compartments, a strong force contributing to (7) can be the net outward transport of fluid by cells (arrows). The resulting pressure difference tends to collapse the interior. *c*: (8) Cells respond to an environmental signal (*) by transporting fluid inwards, causing inflation into an alveolar form.

only lumps and 'brain coral' instead of the more penetrating structures. The depth of the gel was significant, as was the mixing procedure. The initiation of tubular outgrowths seemed in general to be promoted by irregularities in the cell monolayer, including lumps and especially foci of elongated cells.

It is the formation of hollow, branching tubules in these cultures which seems particularly interesting. Mesenchyme may control the initial determination of epithelium as mammary tissue, and can certainly modulate the epithelial branching pattern^{3,4}, but this study shows that the basic 'glandular' form of branching tubes can be generated by isolated, cloned epithelial cells. (The same form is seen in developing salivary gland, pancreas and lung.) This is not too surprising, for Gierer has shown that in theory only one cell type is needed to form tubes, among other structures¹¹. Figure 3 illustrates this with a combination of properties which could plausibly be possessed by the lining epithelial cells of the breast, and also by Rama 25 cuboidal cells. Property (7) may be the least plausible, but glandular epithelial cells contain at their apical or inner face a 'terminal web' of microfilaments which contain myosin-like protein¹² and which may thus be contractile. Mammary ducts and ductules are not always occluded¹³, but morphogenesis can depend on transient processes¹¹. Property (7a) would explain the production of 'domes' or multicellular blisters in cultured sheets of mammary epithelial cells^{7,14}, for domes seem to result from transport of ions and water from the apical to the basal ('outer') cell surface^{14,15}. Milk secretion could provide property (8). The example in Fig. 3 is not meant as a claim that glands contain only one cell type, nor to account for all of mammary morphogenesis. For example it does not explain the branching of ducts. The essential point is that both ducts and alveoli have simple, easily defined forms: forms which do not require multiple cell types.

In conclusion, either the morphogenesis of branching tubules can be achieved by a single epithelial cell population, or

Rama 25 cuboidal cells can produce all the cell types required.

I thank R. Hallowes for valuable advice and discussion; H. Durbin, K. Miller and B. Armstrong for assistance; R. Dulbecco, H. Battifora and D. Schubert for helpful criticism of the manuscript and B. Lang for typing it. This work was supported by the Imperial Cancer Research Fund, London and by grant DRG 254-F from the Damon Runyon Cancer Fund.

Received 29 October 1979; accepted 8 April 1980.

1. Kay, R. R., Town, C. D. & Gross, J. D. *Differentiation* **13**, 7-14 (1979).
2. Mayer, G. & Klein, M. in *Milk: the Mammary Gland and its Secretion* 1 (eds Kon, S. K. & Cowie, A. T.) 47-126 (Academic, New York, 1961).
3. Kratochwil, K. *Dev. Biol.* **20**, 46-71 (1969).
4. Sakakura, T., Nishizuka, Y. & Dawe, C. J. *Science* **194**, 1439-1441 (1976).
5. Yang, J. et al. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3401-3405 (1979).
6. Michalopoulos, G. & Pitot, H. C. *Exp. Cell Res.* **94**, 70-78 (1975).
7. Bennett, D. C., Peachey, L. A., Durbin, H. & Rudland, P. S. *Cell* **15**, 283-298 (1978).
8. Lennon, V. A., Unger, M. & Dulbecco, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6093-6097 (1978).
9. Rudland, P. S., Bennett, D. C. & Warburton, M. J. *Cold Spring Harb. Symp. Cell Proliferation* **6**, 677-699 (1979).
10. Emerman, J. T., Enami, J., Pitelka, D. R. & Nandi, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4466-4470 (1977).
11. Gierer, A. Q. *Rev. Biophys.* **10**, 529-593 (1977).
12. Puchtler, H., Waldrop, F. S., Carter, M. G. & Valentine, L. S. *Histochemistry* **40**, 281-289 (1974).
13. Hollman, K. H. in *Lactation: A Comprehensive Treatise* 1 (eds Larson, B. L. & Smith, V. R.) 3-95 (Academic, New York, 1974).
14. McGrath, C. M. *Am. Zool.* **15**, 231-236 (1975).
15. Cerejido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A. & Sabatini, D. D. *J. Cell Biol.* **77**, 853-880 (1978).

Selective killing of mycoplasmas from contaminated mammalian cells in cell cultures

Menashe Marcus*, Uri Lavi*[†], Alona Nattenberg*, Shlomo Rottem[†] & Ora Markowitz[†]

*Department of Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel

[†]Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Mycoplasmas are common contaminants of animal cells in cell cultures¹⁻³. About 25 *Mycoplasma* and *Acholeplasma* species have been identified as cell culture contaminants but the most frequent are *Mycoplasma hyorhinis*, *Mycoplasma orale*, *Mycoplasma arginini* and *Acholeplasma laidlawii*³. These species are responsible for almost 85% of the contaminations in cell cultures³. We have devised a method for the selective killing of mycoplasmas in contaminated cell cultures, based on the differential nucleic acid metabolism of mycoplasma and mammalian cells. Mycoplasmas are unusual in that they have a nutritional requirement for nucleic acid precursors which can be met by purines and pyrimidine bases or by nucleosides⁴⁻⁶. Mammalian cells, on the other hand, incorporate very little free pyrimidines^{7,8} and for that reason incorporation of free bases such as uracil has been used to detect mycoplasmas in contaminated cell cultures⁹. One of the free base analogues which can be incorporated selectively into nucleic acids of mycoplasmas is 5-bromouracil (5-BrUra). Visible light induces breaks in 5-BrUra-containing DNA¹⁰ and this photosensitivity can be greatly increased by binding of the fluorochrome 33258-Hoechst to DNA¹¹. Its unusually high content of A + T makes the mycoplasma DNA³ an excellent candidate for the induction of breakage by the combined action of 5-BrUra, 33258-H and light because 33258-H has a high affinity for A-T base pairs and an even higher affinity for A-BrUra base pairs¹². We report here that treating them in this way provides a practical and simple method for the selective killing of contaminating mycoplasmas; they are probably killed by the breakage of their DNA.

[†]Permanent address: Agriculture Research Organization, Volcani Center, Bet Dagan, Israel.

Table 1 Incorporation of pyrimidine bases into nucleic acids of uncontaminated and mycoplasma-contaminated mouse RAG cells

³ H-Pyrimidine	Radioactivity (c.p.m. per 10 ⁴ RAG cells)	
	Uncontaminated	Contaminated
Uracil	340	78,000
Thymine	630	550
5-Bromouracil	190	6,800

Uncontaminated or contaminated cells (5×10^4) were inoculated into 3-cm Petri dishes in Dulbecco's modified minimal essential medium (DMEM) enriched with 10% fetal calf serum (FCS). After 20 h of incubation at 37°C the medium was replaced with fresh DMEM containing $1 \mu\text{Ci ml}^{-1}$ (20 Ci mmol^{-1}) of ³H-uracil, ³H-thymine or ³H-5-BrUra. After 60 min of pulse, the cells were trypsinized, washed in cold DMEM and 10^4 cells precipitated in 5% cold trichloroacetic acid (TCA). The precipitate was collected on glass fibre GF/C filters, washed, dried and counted in a Packard Tricarb liquid scintillation spectrometer.

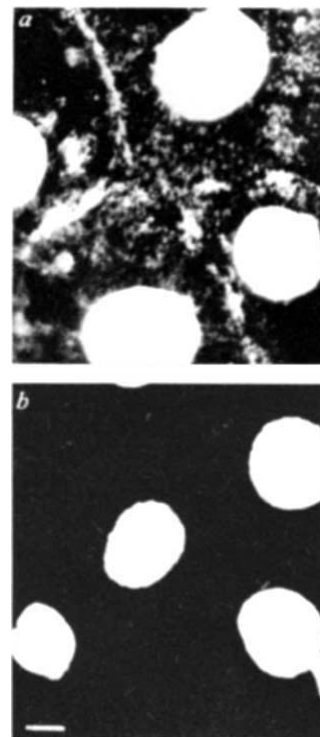
Cells of the Chinese hamster cell line E-36, in small culture flasks containing about 2×10^6 cells, were inoculated with about 10^6 colony-forming units (CFU) of *M. hyorhinis* or *A. laidlawii*. After several weeks of growth, the cell cultures reached a stable state of contamination. Cytological observation by the method of Chen¹³ showed that every cell was contaminated with at least 100 mycoplasma cells. We also used two other cell lines contaminated with unknown mycoplasma strains: one, BHK-21 (ref. 14) originated from the Syrian hamster and the other, RAG (ref. 15) originated from the mouse. Because the mouse cells were very heavily contaminated, we used them to examine the differential incorporation of pyrimidine bases into nucleic acids in both mycoplasma-free and contaminated cells.

Table 1 shows that the incorporation of uracil and 5-BrUra was high in the contaminated cells and very low in mycoplasma-free cells. However, as McIvor and Kenny have shown⁶, thymine incorporation was always low. Similar results were obtained with E-36 cells contaminated with either *M. hyorhinis* or *A. laidlawii*.

The high and selective incorporation of 5-BrUra into contaminated cells encouraged us to treat the cells with the combination of 5-BrUra, 33258-H and light. Treated cells were diluted and clones from single or 100 cells were grown and tested for the presence of mycoplasma. The results of the treatment of E-36 cells contaminated with *M. hyorhinis* are given in Table 2. They show that after 5 days of treatment, E-36 clones grown from 100 cells were cured whereas clones from single cells were cured in 2–3 days (data not shown). Cold thymidine (10^{-5} M) was always added to the cloned cultures to prevent incorporation into cellular DNA of any 5-bromo-2-deoxyuridine (5-BUDr) released from dead mycoplasma cells. 2-Deoxycytidine (10^{-5} M) was also added to the cloned cultures to prevent possible toxic effects of 5-BUDr on the growth of cells¹⁶.

The remaining viable mycoplasma contaminants in the growing clones were detected by selective ³H-uracil incorporation⁹ which is a simple and fast method, by cytological observation¹³ and by the viability counting of CFU⁵. As reported¹⁷, the first two approaches detect only heavy contamination, whereas the CFU technique detects much lower levels of contamination and that was always used as the critical test. We obtained equally good selective killing of mycoplasma from E-36 cells contaminated with *A. laidlawii*. We also eliminated the unknown strains of mycoplasma from RAG cells and BHK-21 cells. The results in Fig. 1 show the effectiveness of our treatment on heavily contaminated RAG cells.

Our findings show that *M. hyorhinis* and *A. laidlawii* contaminating Chinese hamster cells can be selectively killed by our technique and cured clones of cells can be easily obtained from the treated cell culture. The same selective killing technique proved efficient in curing RAG and BHK-21

**Fig. 1** *a*, RAG cells heavily contaminated with mycoplasmas. The cell nuclei have been stained according to Chen¹³. They fluoresce brightly. *b*, Cured RAG cells. No mycoplasma cells are seen. Only brightly fluorescing cell nuclei are seen.**Table 2** Elimination of *M. hyorhinis* from E-36 cells by combined 5-BrUra, 33258-Hoechst and light treatment

5-BrUra treatments (d)	³ H-Uracil incorporation (c.p.m. per 10 ⁴ E-36 cells)	Cytological determination of contaminants	Viability count (CFU ml ⁻¹)
0	31,000	++++	2×10^7
2	700	++	1.8×10^5
3	190	+	4×10^3
4	220	—	1×10^2
5	70	—	0

Contaminated cells were grown at 37°C in DMEM + FCS. 5-BrUra ($30 \mu\text{g ml}^{-1}$) was added to the cultures. After 24 h 33258-Hoechst ($1 \mu\text{g ml}^{-1}$) was added for 60 min and the cultures were then illuminated by a 400-W cold bright fluorescence lamp at a distance of 5 cm for 30 min. The medium was then replaced with a fresh DMEM + FCS containing 5-BrUra and the Hoechst treatment was repeated at 24-h intervals. After two to five treatments the cells were trypsinized, washed and diluted in fresh DMEM + FCS containing 10^{-5} M thymidine and 10^{-5} M 2-deoxycytidine. The cells were cloned in Microtest plates. In each well, clones from 100 cells were started. The growing clones were transferred to 3-cm Petri dishes and screened for mycoplasma contamination by the DNA fluorochrome staining procedure¹³ and the CFU counting technique⁵. ³H-Uracil incorporation into acid-insoluble material was determined 24 h after the end of each combined 5-BrUra-33258-Hoechst-light treatment. E-36 cells (5×10^4) were inoculated into a 3-cm Petri dish in DMEM + FCS. After 24 h of incubation at 37°C the medium was replaced with fresh DMEM containing ³H-uracil ($1 \mu\text{g ml}^{-1}$, 20 Ci mmol^{-1}). After 60 min the cells were trypsinized, washed in cold DMEM and 10^4 cells were precipitated in 5% cold TCA. The precipitate was collected by filtration, washed, dried and counted as in Table 1. Cytological determinations were carried out according to Chen¹³ using the DNA fluorochrome staining procedure. The results are expressed as follows: 4+, all cells are contaminated with at least 100 mycoplasmas per cell; 2+, only some of the cells are contaminated with 5–50 mycoplasmas per cell; +, only a few cells are contaminated with 1–20 mycoplasmas per cell; —, no mycoplasma cells observed in at least 50 screened E-36 cells.

cells from unknown contaminating mycoplasma strains. Our cured RAG and BHK-21 clones have already been grown continuously for several months and no contaminating mycoplasma has been found. These results suggest that our technique could be applied generally in the elimination of contaminating mycoplasma strains from mammalian cell cultures.

We thank Dr H. Loewe (Hoechst) for the gift of 33258-H. This investigation was supported by a grant from the A. D. Bergman Science Foundation no. 015-6933 to M.M. and S.R. and a grant from the US-Israel Binational Science Foundation no. 1962/79, to M.M.

Received 28 February; accepted 28 April 1980.

1. Stanbridge, E. *Bact. Rev.* **35**, 206–227 (1971).
2. Barile, M. F. in *Contamination in Tissue Culture*, 131–172 (Academic, New York, 1973).
3. Barile, M. F. in *The Mycoplasmas* (eds Tully, J. G. & Whitcomb, R. F.) 425–474 (Academic, New York, 1979).
4. Rodwell, A. W. J. *gen. Microbiol.* **58**, 39–47 (1969).
5. Stanbridge, E. J., Haylick, L. & Perkins, F. T. *Nature new Biol.* **232**, 242–244 (1971).
6. Melvor, R. S. & Kenny, G. E. *J. Bact.* **135**, 483–489 (1978).
7. Prusoff, W. H. *Cancer Res.* **23**, 1246–1259 (1963).
8. Levine, E. M. & Becker, B. G. in *Mycoplasma Infection of Cell Cultures* (eds McGarrity, G. J., Murphy, D. G. & Nichols, W. W.) 87–104 (Plenum, New York, 1978).
9. Kenny, G. E. in *Microbiology* (ed. Schlesinger, D.) 32–36 (American Society for Microbiology, Washington, 1975).
10. Puck, T. T. & Kao, F. T. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1227–1234 (1967).
11. Stetten, G., Latt, S. A. & Davidson, R. L. *Somatic Cell Genet.* **2**, 285–290 (1976).
12. Latt, S. A. & Wohlleb, J. C. *Chromosoma* **52**, 297–316 (1975).
13. Chen, T. R. *Expl Cell Res.* **104**, 255–262 (1977).
14. MacPherson, T. & Stoker, M. *Virology* **16**, 147–151 (1962).
15. Klebe, R. J., Chen, T. R. & Ruddle, F. H. *J. Cell Biol.* **45**, 74–82 (1970).
16. Meuth, M. & Green, H. *Cell* **2**, 109–112 (1974).
17. Thangavelu, M., Ernp, H. & Therkelsen, A. J. *Hum. Genet.* **47**, 199–202 (1979).

Macrophages overcome mycoplasma infections of cells *in vitro*

L. Schimmelpfeng, U. Langenberg & J. Hinrich Peters

Institut für Genetik, Universität Köln, Weyertal 121, D-5000 Köln 41, FRG

Mycoplasma infections still cause severe problems in cell cultures, particularly permanent lines, and although rapid detection is possible^{1,2} the only methods proposed for the elimination of the mycoplasma are either laborious or unsatisfactory. Treatment with antibiotics often leads to the development of resistance³ and we have found it more successful to passage contaminated cells in nude (thymusless) mice⁴ although the cells cannot always be recovered. But when the resulting subcutaneous tumours can be collected, the cells are both free of mycoplasma and accompanied by a large number of macrophages. Because nude mice have no T cell-dependent immune response, it seemed possible that the macrophages could be responsible for the elimination of the mycoplasma. The experiments reported here support this hypothesis, and have led to a rapid and reproducible technique for eliminating mycoplasma *in vitro* by a brief co-cultivation of contaminated cells with mouse macrophages, in the presence of antibiotics.

Three different preparations of mouse macrophages were used with identical success: peritoneal macrophages actuated by thioglycollate, the same preparation kept as frozen stock before use (frozen in 90% serum plus 10% dimethyl sulphoxide), and macrophages grown *in vitro* from bone marrow precursors stimulated with conditioned medium containing macrophage growth factor⁵. Eradication of mycoplasma was most efficient when contaminated cells were applied to a macrophage monolayer in a ratio of about 1:100, together with antibiotics (Table 1). If the ratio of cells to macrophages was greater or if either antibiotics or macrophages were omitted, results were less reliable, although

in the absence of antibiotics macrophages sometimes eliminated the mycoplasma. Antibiotics alone, as expected, were variably effective. Conditioned medium obtained from thioglycollate-activated macrophages did not affect contamination nor markedly alter the effect of antibiotics.

To confirm these results, we co-cultivated 10 permanent cell lines (BHK, RBH, NRK, 3T3, L6TG, L929, A9, A9HT, D-98AH2, and KB) heavily infected with mycoplasma, with macrophage monolayers (infected cells/macrophages 1:100), in the presence of antibiotics. When cells grew particularly rapidly on the macrophage monolayer (probably due to a feeder layer effect), we reduced the serum concentration to 2%, in order to slow down the proliferation and not to exceed the optimal cell number. Following this protocol, we purified all cell lines. Most of them have been examined repeatedly for

Table 1 Effect of macrophages, macrophage-conditioned medium and antibiotics on mycoplasma elimination from two cell lines

Treatment	Cell type	No. of cells inoculated ($\times 10^{-3}$)					
		1	2	4	8	10	14
Antibiotics	A9	—	—	+	+	+	+
		+	+	+	+	+	+
	BHK	—	—	—	—	—	—
		—	—	—	—	—	—
Conditioned medium	A9	+	+	+	+	+	+
		+	+	+	+	+	+
	BHK	+	+	+	+	+	+
		+	+	+	+	+	+
Conditioned medium plus antibiotics	A9	+	+	+	+	+	+
		—	—	—	—	—	—
	BHK	—	—	—	+	+	+
		—	—	—	+	+	+
Macrophages (3.5×10^5 per well)	A9	+	+	+	+	+	+
		+	+	+	+	+	+
	BHK	—	—	+	+	+	+
		—	—	—	—	+	+
Antibiotics plus macrophages	A9	—	—	—	—	+	+
		—	—	—	—	+	+
	BHK	—	—	—	—	—	—
		—	—	—	—	—	—

Mouse peritoneal macrophages were elicited by intraperitoneal injection of 1 ml 10% thioglycollate medium (Difco 0256-01) into BALB/c mice. Four days later the cells were collected by rinsing of the peritoneal cavity and cultured at 3.5×10^5 cells per 1.5-cm well in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% heat inactivated fetal calf serum, penicillin ($10,000 \text{ U ml}^{-1}$) and streptomycin ($10,000 \mu\text{g ml}^{-1}$). After 1 day of culture, the cells had formed a confluent monolayer. Non-adherent cells were removed by rinsing with growth medium. Mycoplasma-infected cells, either mouse A9 fibroblasts (a derivative of mouse L cells), or baby hamster kidney (BHK) cells were seeded at various numbers onto the macrophage monolayers. Control cultures contained no macrophages. Some of the cultures received mycoplasma-specific antibiotics ($100 \mu\text{g ml}^{-1}$ lincomycin plus $100 \mu\text{g ml}^{-1}$ tylosin), and/or 50% conditioned medium from 2 days cultures of thioglycollate elicited macrophages. After 3 days, A9 or BHK cells were separated from macrophages by treatment with trypsin (0.2%), a dose which does not detach macrophages. After another 3 days of culture, recovered cells were tested for the presence of mycoplasma by staining with the DNA-specific fluorescent dye, Hoechst 33258, previously described as a mycoplasma test¹. After another 2–4 weeks of culture, the cells were examined by autoradiography of incorporated tritiated thymidine ($1 \mu\text{Ci}$ per ml per 24 h; 25 Ci mmol^{-1} , exposure time 4 days). In uninfected cells labelling was restricted to the nucleus, whereas mycoplasma-infected cells showed a marked peripheral labelling⁶. The results are expressed as presence (+) or absence (—) of mycoplasma. Double symbols indicate the results of two independent experiments.

more than 6 months and no mycoplasma recrudescence has been observed.

Because macrophage-conditioned medium did not affect the mycoplasma, we do not regard secreted macrophage products (such as lysozyme) as the principal factors acting against mycoplasma. Instead, macrophages seem to act directly on the mycoplasma, particularly by phagocytosis. The combined action of phagocytic macrophages and antibiotics may reduce the probability that antibiotic resistance will develop, for macrophages rapidly clear the cultures of contaminants. However, we cannot rule out the possibility that co-cultivation may carry the risk of infecting the cells with endogenous murine viruses from the macrophages, as is equally true for cells injected into nude mice.

The observed synergism of macrophages and antibiotics may be useful for the *in vitro* study of defence mechanisms that are influenced by antibiotics *in vivo*. Indeed, we have observed the complete elimination of yeast and bacteria from tissue culture cells through the combined action of macrophages and antibiotics (kanamycin, gentamycin, or nystatin) when antibiotic treatment in the absence of macrophages is of limited efficacy.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 74).

Received 11 February; accepted 26 March 1980.

1. Russel, W. C., Newman, C. & Williams, D. H. *Nature* **253**, 461-462 (1975).
2. McGarrity, G. J., Vanaman, V. & Sarama, J. *Expl Cell Res.* **121**, 159-165 (1979).
3. Kenney, G. E. *In Vitro* **14**, 338 (1978).
4. Van Diggelen, O. P., Shin, S. & Philipps, D. M. *Cancer Res.* **37**, 2680-2687 (1977).
5. Meerpohl, H. G., Lohmann-Matthes, M. L. & Fischer, H. *Eur. J. Immun.* **6**, 213-217 (1976).
6. Perez, A. G., Kim, J. H., Gelbard, A. S. & Djordjevic, B. *Expl Cell Res.* **70**, 301-310 (1972).

In vivo cyclic change in B-lymphocyte susceptibility to T-cell control

R. A. Calderon & D. B. Thomas

Division of Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

The humoral response to hapten-protein conjugates is an invaluable model for dissecting the cellular elements of lymphocyte cooperation, and the Mitchison secondary adoptive transfer system provides convincing evidence of cooperation between hapten-specific B cells and carrier-specific T cells in the production of anti-hapten antibody¹⁻³. Recently, attention has focused on the role of suppressor T cells in the regulation of antibody production⁴. Several workers have shown that carrier-priming may, in some instances, suppress a subsequent hapten antibody response, both *in vivo* and *in vitro*⁵⁻⁹. This effect is attributed to a suppressor T-cell population, generated during the initial phase of the immune response^{8,9}. Gershon and co-workers have postulated that such suppressor T cells function in a feedback regulatory loop to limit the duration of an immune response¹⁰. We have examined the suppressive effect of carrier immunization in a secondary anti-hapten response *in vivo* and demonstrate a cyclic change in susceptibility of memory B cells to T-help and suppression. Such variation presents a severe restriction to any model of feedback control by suppressor T cells.

Spleen cells from CBA mice, primed 2-4 months earlier to dinitrophenyl keyhole-limpet haemocyanin (DNP-KLH), were adoptively transferred to lethally irradiated syngeneic recipients and assayed for indirect, anti-hapten plaque forming

cells (IPFC) at day 6 (Table 1). Treatment of the donor population with anti-Thy-1 and complement eliminated the IPFC response and this could be restored to almost normal values by the supplementary transfer of KLH-primed spleen cells (Table 1, expt 1b, c). There was no detectable anti-hapten response, however, if the carrier-primed population was boosted with KLH 3 days before cell transfer (expt 1d). This was unlikely to be a trivial artefact, such as failure of 'activated' helper cells to localize in recipient spleens, as boosting of hapten-primed donors with DNP-KLH (expt 2a) or KLH alone (expt 2b) did not diminish a subsequent anti-hapten response. Lack of cell cooperation was also observed in a heterologous system using DNP-KLH-primed spleen cells and human γ -globulin (HGG)-primed, or boosted, helper cells (expt 3) and the control experiment (expt 4) ruled out any nonspecific effect of immunization: HGG-boosted spleen cells did not suppress a secondary response to DNP-KLH.

There was a brisk decline in helper activity of carrier-primed donors after re-challenge with KLH (Fig. 1a), reaching a baseline value at 3 days followed by a gradual recovery to the pre-boost level. A comparison was also included of the effects of carrier-boosting in adult-thymectomized donors (as thymectomy is claimed to diminish suppressor T-cell activity¹¹⁻¹³). Here, the effects of carrier immunization were marginal.

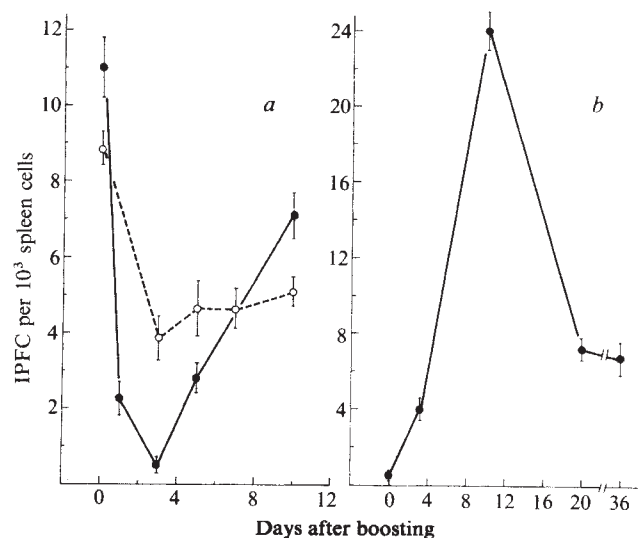


Fig. 1 *a*, Effect of carrier-boosting at various times before adoptive transfer. Groups of five normal, or adult-thymectomized CBA mice, primed to KLH, were injected i.v. with aqueous antigen 10 μ g at the times indicated, before adoptive transfer. Spleen cells (10^7) were combined with DNP-KLH-primed B cells (10^7 , previously treated with anti-Thy-1 and C'), 10 μ g DNP-KLH and transferred to irradiated recipients. Adult thymectomy was carried out at 4 weeks and mice were primed with KLH alum-pertussis 1 week later. ●, Normal, KLH-primed mice; ○, adult thymectomized, KLH-primed mice. *b*, Effect of hapten-carrier boosting at various times before adoptive transfer. Groups of five CBA mice, primed to DNP-KLH, were injected i.v. with aqueous DNP-KLH (10 μ g), at the times indicated, before adoptive transfer. Spleen cells were treated with anti-Thy-1 and C', and combined with KLH-primed spleen cells (10^7), boosted 3 days earlier with KLH (10 μ g).

It could be argued from the above, and earlier reports of carrier-specific suppression in primary anti-hapten responses⁷⁻⁹, that carrier immunization recruits a population of suppressor cells during the initial phase (Fig. 1a; 3 days) of the immune response. However, a strikingly different picture emerged when helper activity was assayed against memory B cells which had also been re-challenged with antigen before adoptive transfer (Fig. 1b): KLH-primed spleen cells, boosted

Table 1 Effect of carrier boosting on a secondary anti-hapten response *in vivo*

Hapten-primed donor	Carrier-primed donor	Antigen	IPFC per 10 ⁶ spleen cells*
Expt 1			
a DNP-KLH	---	DNP-KLH	11,089 ± 750
b DNP-KLH(anti-Thy-1 + C')†	---		< 150
c DNP-KLH(anti-Thy-1 + C')†	KLH		3,770 ± 253
d DNP-KLH(anti-Thy-1 + C')†	KLH (-3d)‡		< 150
Expt 2			
a DNP-KLH + hapten carrier (-3d)‡	---	DNP-KLH	8,443 ± 770
b DNP-KLH + carrier (-3d)‡	---		2,721 ± 330
c DNP-KLH + carrier (-3d)‡ (anti-Thy-1 + C')	KLH (-3d)‡		178 ± 30
Expt 3			
a DNP-KLH	---	DNP-HGG	2,915 ± 216
b DNP-KLH	HGG		21,008
c DNP-KLH(anti-Thy-1 + C')	HGG		24,022 ± 2,129
d DNP-KLH	HGG (-3d)‡		340 ± 64
e DNP-KLH(anti-Thy-1 + C')	HGG (-3d)‡		1,393 ± 177
Expt 4			
a DNP-KLH	---	DNP-KLH	59,400 ± 5,400
b DNP-KLH(anti-Thy-1 + C')	KLH (-3d)‡		750 ± 105
c DNP-KLH	HGG (-3d)‡		57,383 ± 7,779

CBA/Ca mice were immunized at 6–8 weeks with 100 µg alum-precipitated antigen (DNP₂₇₀-KLH; KLH; DNP₁₆-HGG; HGG) and 10⁹ *Bordetella pertussis* and used 2–4 months later. Secondary challenges (boosting) of aqueous antigen (10 µg) were injected intravenously (i.v.). Recipients were irradiated with 850 rad from a ⁶⁰Co source and injected i.v. a few hours later with 10⁷ hapten-primed spleen cells, and/or 10⁷ carrier-primed spleen cells, and aqueous antigen (10 µg).

*Indirect plaque-forming cells (PFC) were assayed by the slide method¹⁷, using DNP-Fab-anti-sheep red blood cells to coat erythrocytes, and developed with a polyspecific rabbit anti-Ig in the presence of a specific goat anti-µ serum (to suppress direct PFC). Values for IPFC per 10⁶ spleen cells are the arithmetic mean ± s.e.m. for three or more spleens, assayed individually at two or more cell dilutions.

†Spleen cells (4 × 10⁸, 2 ml) were incubated for 30 min at 3 °C with an equal volume of anti-Thy-1.2 serum (¼ dilution; AKR anti-C3H, cytotoxic titre > 1/200), washed and reincubated for 30 min at 37 °C with guinea pig complement (agarose absorbed; ⅓ dilution).

‡DNP-KLH or KLH or HGG-primed mice were boosted with 10 µg antigen 3 days before adoptive transfer.

3 days before adoptive transfer, suppressed a secondary anti-DNP response yet gave adequate help to memory B cells, re-challenged with antigen, 3–36 days before transfer.

There were several likely reasons for these cyclic changes in the anti-hapten response after boosting of the carrier (Fig. 1a) or hapten-primed donors (Fig. 1b). For instance, a secondary response to carrier might alter a pre-existing homeostatic balance between helper cells and suppressor cells in favour of the latter and/or produce a quantitative reduction in effective T-cell help. Alternatively, cell cooperation might require synchronous recognition of antigen by B and T cells, and subsequent cell proliferation leading to a parallel decline in the help provided (Fig. 1a) and help required (Fig. 1b) on adoptive transfer. The following cell titration experiments show that carrier immunization generates a population of suppressor T cells, whereas boosting of memory cells does not alter their requirement for carrier-specific help.

Optimum anti-DNP responses (5–15 × 10⁵ IPFC per spleen) were regularly obtained on adoptive transfer to irradiated recipients of 10⁷ DNP-KLH-primed B cells and 0.6–0.8 × 10⁷ KLH-primed spleen cells. Addition of increasing numbers of KLH-primed and boosted (-3 days) spleen cells led to a progressive decline in IPFC, with optimum suppression (>90%) at cell ratios >1.5 for carrier-boosted to carrier-primed cells (Fig. 2). Suppression was reduced by pretreatment of the carrier-boosted population with anti-Thy-1 and complement. In a reciprocal titration, optimum help was obtained at cell ratios >1.5 for carrier-primed to carrier-boosted donor cells. These results suggest that help or suppression in a secondary anti-hapten response is determined by the numerical balance between two populations of carrier-specific T cells; in which case there is the immediate paradox that memory B cells, re-challenged with antigen, cooperate superbly with a carrier-primed (and boosted, -3 days) population containing an apparent excess of suppressor cells (Fig. 1b). This suggests that memory cells become refractory to T-cell suppression, and/or require less 'available' help after

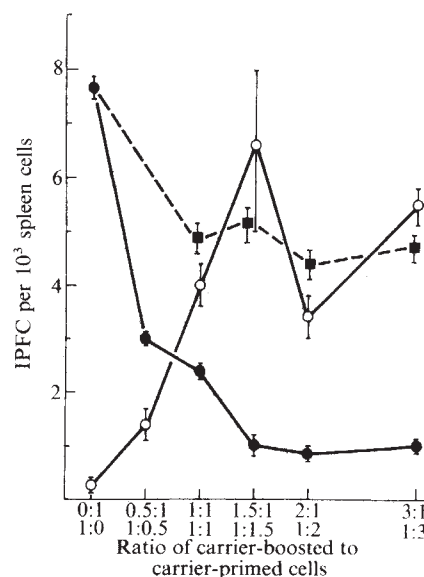


Fig. 2 Suppression of a secondary anti-DNP response by various proportions of carrier-primed to carrier-boosted spleen cells. ●, 10⁷ DNP-KLH-primed B cells, 6 × 10⁶ KLH-primed spleen cells and increasing proportions (6–18 × 10⁶) of KLH-boosted (3 days previous) spleen cells. ○, 10⁷ DNP-KLH-primed B cells, 6 × 10⁶ KLH-boosted (3 days previous) spleen cells and increasing proportions (6–18 × 10⁶) of KLH-primed spleen cells. ■, 10⁷ DNP-KLH-primed B cells, 6 × 10⁶ KLH-primed spleen cells and increasing proportions of KLH-boosted (3 days previous) spleen cells, pretreated with anti-Thy-1 and C'. Cell numbers were adjusted to equivalence before cell transfer (3.4 × 10⁷ per recipient) by the appropriate addition of normal spleen cells.

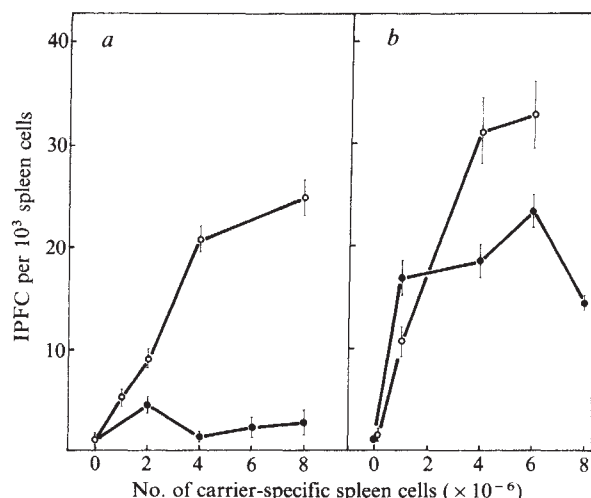


Fig. 3 Titration of carrier-specific help for: *a*, DNP-KLH primed B cells; *b*, DNP-KLH primed and boosted (10 days previous) B cells. ○, KLH-primed spleen cells; ●, KLH-primed and boosted (3 days previous) spleen cells. Cell numbers were adjusted to equivalence before cell transfer (18×10^6 per recipient) by the appropriate addition of normal spleen cells.

secondary challenge. To solve this problem, a limiting dilution analysis was made of the helper requirements for memory cells before, and after, a secondary antigenic challenge.

Constant numbers of DNP-KLH-primed B cells (10^7) were transferred to lethally irradiated recipients together with limiting numbers of KLH-primed, or boosted (–3 days), spleen cells ($1-8 \times 10^6$) and assayed for an anti-DNP response. Increasing the number of carrier-primed cells produced a linear increase in the IPFC response to a discrete plateau ($6-8 \times 10^6$, slope of 1 for log dose–response curve), whereas a background plaque response was obtained at all cell dilutions for the carrier-boosted donors. (Janeway¹⁴ has claimed a ‘premium effect’ in this assay system with increasing numbers of helper cells, indicative of 2-hit kinetics, but we have consistently failed to confirm that finding.)

In a parallel experiment, B cells were re-challenged with DNP-KLH 10 days before adoptive transfer. There was no significant reduction in the requirement for help from carrier-primed donors (Fig. 3*b*), but surprisingly the carrier-boosted populations cooperated optimally at much lower cell dilutions ($1-4 \times 10^6$). Once again, the paradox is emphasized: a carrier-specific population, containing an apparent excess of suppressor cells (Figs 1*a*, 3*a*) provided adequate help for memory B cells also re-challenged before adoptive transfer (Figs 1*b*, 3*b*).

If carrier-specific help and suppression (Table 1) are indeed mediated by distinct T-cell subsets¹⁵, an abrupt and qualitative change occurs in the B-cell memory compartment after antigenic challenge, rendering the population refractory to T-cell suppression (Figs 1*b*, 3*b*). North and Askonas have reported a qualitative change in memory B cells after secondary challenge, as measured by their ability to sustain an IgG anti-DNP response *in vitro*¹⁶. Elson and Taylor have already proposed that in a hapten-carrier system ‘help or suppression depends on the stage of progression of T-cells in relation to the stage of progression of B-cells in their respective responses’ (ref. 7).

A temporal restriction of T-cell help and suppression, imposed by the B cell, would provide a first order control to the immune response, ensuring selective and synchronous recruitment of memory B cells. Cyclic changes in the susceptibility of a B cell to T-cell help and suppression affords a mechanism, albeit in a hapten-carrier secondary adoptive transfer system. Perhaps the most significant finding presented

here is that the suppressor cells, generated by carrier immunization, exert no effect on an ongoing anti-hapten response, but rather may prevent its initiation. This raises an immediate question: how do suppressor T cells function in the postulated feedback regulatory loop¹⁰ to curtail an immune response? The paradox is still to be resolved.

We thank Miss Lynda Blaxland and Miss Christine Graham for technical assistance. R.A.C. acknowledges financial support from Consejo Nacional de Ciencia y Tecnología, Mexico.

Received 22 January; accepted 28 March 1980.

1. Ovary, Z. & Benacerraf, B. *Proc. Soc. exp. Biol. Med.* **114**, 72–76 (1963).
2. Mitchison, N. A. *Eur. J. Immun.* **1**, 18–27 (1971).
3. Raff, M. C. *Nature* **226**, 1257–1258 (1970).
4. Gershon, R. K. *Transplant. Rev.* **26**, 170–185 (1975).
5. Katz, D. A., Paul, W. E. & Benacerraf, B. *J. Immun.* **110**, 107–117 (1973).
6. Tada, T. & Takemori, T. *J. exp. Med.* **140**, 239–252 (1974).
7. Elson, C. J. & Taylor, R. B. *Eur. J. Immun.* **4**, 682–687 (1974).
8. Eardley, D. D. & Sercarz, E. E. *J. Immun.* **116**, 600–605 (1976).
9. Eardley, D. D. & Sercarz, E. E. *J. Immun.* **118**, 1306–1310 (1977).
10. Eardley, D. D. *et al.* *J. exp. Med.* **147**, 1106–1115 (1978).
11. Baker, P. J., Stashak, P. W., Ansbaugh, D. F., Prescott, B. & Barth, R. F. *J. Immun.* **105**, 1581–1583 (1970).
12. Okumura, K. & Tada, T. *J. Immun.* **106**, 1019–1025 (1971).
13. Basten, A., Miller, J. F. A. P. & Johnson, P. *Transplant. Rev.* **26**, 130–169 (1975).
14. Janeway, C. A. *J. Immun.* **114**, 1394–1401 (1975).
15. Cantor, H. & Boyse, E. A. *J. exp. Med.* **141**, 1376–1389 (1975).
16. North, J. R. & Askonas, B. A. *Eur. J. Immun.* **6**, 8–15 (1976).
17. Dresser, D. W. in *Handbook of Experimental Immunology* 3rd edn (ed. Weir, D. M.) (Blackwell, Oxford, 1978).

Carrier-priming leads to hapten-specific suppression

Leonore A. Herzenberg, Takeshi Tokuhisa & Leonard A. Herzenberg

Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

Studies over the past two decades have established^{1,2} that ‘helper’ T lymphocytes primed to a ‘carrier’ protein increase B-lymphocyte antibody responses to ‘haptenic’ determinants on the carrier protein. T cells from donors primed with one commonly used carrier protein, keyhole limpet haemocyanin (KLH), have been shown by many workers to augment B-cell IgG anti-dinitrophenyl (DNP) responses in adoptive recipients stimulated with DNP-KLH. Therefore, we were surprised recently to find that the KLH-primed mice we normally use as donors for carrier-specific helper T cells in adoptive transfers³ show reduced rather than augmented IgG *in situ* anti-DNP antibody production when stimulated with DNP-KLH. Here we show that hapten-specific regulation of antibody production is responsible for this response failure. That is, that KLH-priming before DNP-KLH exposure reduces the production of IgG anti-DNP antibody without interfering with the anti-KLH response or with the development of anti-DNP memory B cells, and that IgG anti-DNP production remains low after further stimulation with DNP on KLH or an unrelated carrier. Thus initial stimulation with a carrier-protein creates an oddly compromised animal in which IgG antibody production to a ‘new’ haptenic determinant on the carrier is kept minimal despite the presence of normal anti-hapten memory and carrier-specific help capable of supporting other responses to the hapten-carrier conjugate.

Figure 1 shows that very little IgG anti-DNP antibody is produced in KLH-primed mice stimulated with $100 \mu\text{g}$ DNP-KLH on alum and stimulated again 6 weeks later with $1 \mu\text{g}$ of aqueous DNP-KLH. The average affinity of this minimal anti-DNP response is 10–100-fold lower than the anti-DNP affinities obtained in mice not previously exposed to KLH (Table 1). The IgG-1a (IgG2a) anti-DNP responses from

Table 1 Carrier-priming reduces the amount and affinity of anti-hapten responses stimulated by the hapten-carrier conjugate but does not interfere with anti-carrier response

Group	Antigenic stimulation Protocol	Time (weeks)	In situ IgG2a antibody responses		
			Anti-KLH units	Anti-DNP units	Mean K_a ($\times 10^6$)
I	Hapten-carrier first DNP-KLH (100 μ g on alum)	→ 0			
		2	15	35	(5)
		6	15	30	(8)
	DNP-KLH (1 μ g aqueous)	→ 6			
		7	130	140	(100)
II	Carrier first KLH (100 μ g on alum)	→ -6			
		-4	20	<1	(<0.3)
		0			
	DNP-KLH (100 μ g on alum)	→ 2	170	5	(<0.3)
		6	ND	5	(<0.3)
	DNP-KLH (1 μ g aqueous)	→ 6			
		7	370	9	(0.5)
III	Hapten-carrier first DNP-KLH (100 μ g aqueous)	→ 0			
		2	4	3	(<0.3)
IV	Carrier first KLH (100 μ g on alum)	→ -6			
	DNP-KLH (100 μ g aqueous)	→ 0			
		2	140	5	(<0.3)

(BALB/c $\times$ SJL) F_1 hybrid mice (Igh^a/Igh^b), approximately 10 weeks old at the beginning of the stimulation protocols, were used. All antigens were injected intraperitoneally (i.p.). Mice were bled from the tail at indicated times and serum assayed for anti-KLH and anti-DNP activities by solid phase radioimmunoassay (RIA). Data for the Igh-1a (IgG2a) allotype response are shown here; Igh-1b allotype responses were similar. IgG1 responses were somewhat higher (see text). Units of antibody are equal to the percentage of allotype anti-DNP antibody in a standard serum pool of BALB/c $\times$ SJL adoptive secondary response sera^{1,2}; 1 unit $\approx$ 1 μ g ml⁻¹ of anti-DNP antibody. Mean K_a (M⁻¹) values are estimated from the ratio of RIA binding obtained on DNP₁₂ bovine serum albumin (BSA) and DNP₄₂BSA. Studies with monoclonal anti-DNP antibodies have shown that this ratio is proportional to log K_a determined by fluorescence quenching with ϵ -DNP-lysine³. ND, not determined.

Igh^a/Igh^b (BALB/c $\times$ SJL) F_1 hybrids shown are similar to Igh-1b responses in the same mice. Production of IgG₁ anti-DNP is also reduced in carrier-primed mice but often increases with repeated DNP-KLH stimulation. IgM responses, in contrast, are not reduced (data not shown). These findings are representative of results obtained using a variety of protocols in which DNP-KLH was injected 1–6 weeks after KLH and at different doses either on alum or in aqueous form (see Table 1).

Table 1 also shows that although the anti-DNP response is severely reduced in the KLH-primed mice exposed repeatedly to DNP-KLH, the anti-KLH response in these mice proceeds normally (that is, increases with each subsequent exposure to KLH determinants). As both these responses require help from carrier-primed T cells, the specific and continued failure of the anti-DNP response cannot be ascribed simply to the lack of such help. Nor can this failure be ascribed to deficiencies in anti-DNP memory B-cell development. Adoptive transfer data in Table 2 show that splenic memory B cells generated by DNP-KLH in KLH-primed mice (groups I and III) are indistinguishable from memory populations generated by our usual protocol for priming anti-DNP memory B cells (group II)³. Both the magnitude and the affinity of B-cell memory in each of these three groups are essentially the same. Thus functional memory B cells are clearly present but generally remain silent in mice primed first with KLH (on alum) and then exposed repeatedly to DNP-KLH.

In situ and memory responses from mice primed with aqueous DNP-KLH (group III, Tables 1 and 2) show that inadequate priming reduces all responses to the hapten-carrier conjugate rather than specifically reducing anti-DNP antibody production. These results therefore indicate that alum priming

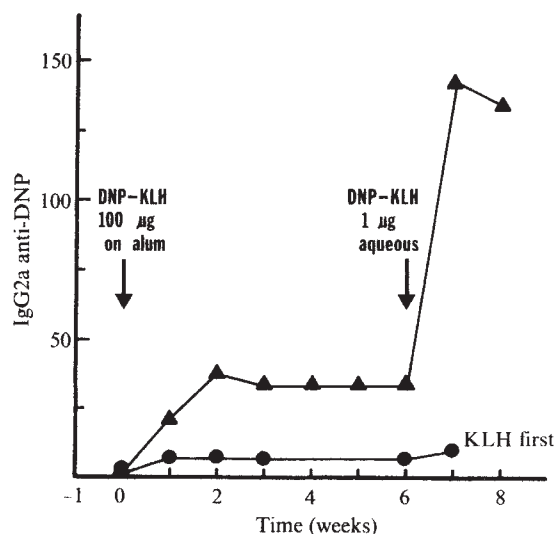


Fig. 1 Carrier-priming prevents hapten-carrier stimulation of anti-hapten antibody responses. (BALB/c $\times$ SJL) F_1 hybrids were used for these experiments. BALB/c and BAB/14 mice showed similar results. Data for Igh-1a (IgG2a) allotype anti-DNP responses are shown here. Protocol and assay details are as described in Table 1 legend. ●, Primed with 100 μ g KLH at -6 weeks; ▲, no prior antigenic exposure.

with KLH (free or hapten-conjugated) is required to induce adequate carrier-specific help for optimal *in situ* antibody production and memory B-cell development. Furthermore, since anti-DNP memory development occurs normally in KLH (on alum)-primed mice exposed to DNP-KLH, these results confirm that adequate carrier-specific help is present and operative with respect to DNP-KLH in these mice, even though the anti-DNP antibody production is minimal.

Finally, data in Fig. 2 show that exposure of KLH-primed mice to DNP-KLH renders these mice incapable of responding to DNP conjugated to an unrelated carrier protein but does not interfere with response to the carrier protein itself, that is chicken γ -globulin (CGG). Figure 2 also shows that mice not exposed to DNP-KLH respond normally to DNP-CGG. Therefore, we conclude first that the induction of non-responsiveness to DNP requires exposure to hapten on the carrier used initially for priming, and second that the effector mechanism responsible for preventing production of the anti-DNP antibody is hapten-specific and does not depend on specific interaction with the carrier protein.

Since the effector mechanism prevents production of antibodies with particular variable region structures (that is the majority of those capable of combining with DNP) and since it 'actively' suppresses anti-DNP responses by co-transferred DNP-primed populations in adoptive transfer recipients (data not shown), we tentatively regard the non-responsiveness for DNP demonstrated here as due to the induction of a broad idiosyncrasy suppression^{4,5} occasioned by exposure of carrier-primed animals to DNP conjugated to the carrier used for priming.

The characteristics of *in situ* antibody responses in a number of other immunization systems indicate that the inability of carrier-primed mice to produce IgG antibody to 'new' haptenic determinants on the carrier is a general regulatory phenomenon which applies even to haptenic determinants presented with the carrier when these initially fail to stimulate an antibody response. For example, we have observed that repeated stimulation of individual mice producing antibodies to IgG2a allotypic determinants elicits production of very little antibody to determinants not detected

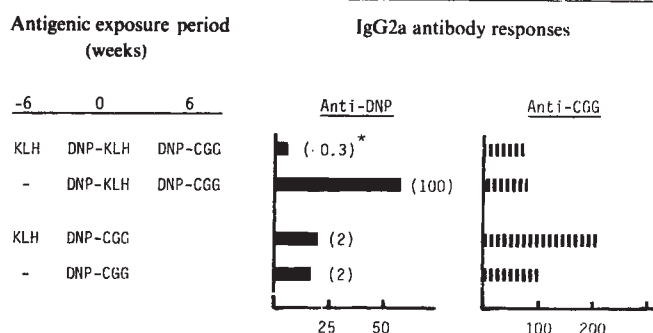


Fig. 2 Homologous hapten-carrier conjugate induces hapten-specific suppression in carrier-primed mice. Each indicated antigenic stimulation was given as 100 μ g of antigen on alum, i.p. Responses were measured 1 week after last indicated stimulation; RIA units shown are relative to 'standard' antiserum pools for each antigen. Mean K_a (in parentheses, $\times 10^6$) was determined from RIA binding. Assay details are as described in Table 1 legend.

in the early antibody populations. "Original antigenic sin" studies⁶ with viral antigens also suggest that initial reactivity defines the scope of subsequent antibody production. Finally, although we (and some of our colleagues) were surprised that carrier-primed mice respond poorly *in situ* to a 'new' hapten on a hapten-carrier conjugate, the basic elements of this finding have already been described^{1,2,7}.

In fact, extensive evidence (albeit otherwise interpreted) has been presented demonstrating that suppression for anti-hapten responses develops when carrier-priming precedes exposure to hapten-carrier conjugates. 'Carrier-specific' suppression, for example⁸, is usually defined by the ability of carrier-primed mice to suppress the response to a hapten conjugated to the priming carrier but not to the same hapten on a non-related carrier. Our demonstration that hapten-carrier conjugates induce specific anti-hapten suppression in similarly primed mice suggests (but does not prove) that in some cases this so-called carrier-specific suppression may really be hapten-specific. A similar argument applies to studies of feedback suppression⁹.

Evidence^{1,2,7} demonstrating that carrier-priming reduces subsequent responses to hapten-carrier conjugates has been largely ignored because hapten and carrier immunization protocols were developed that yielded the expected *in situ* secondary anti-hapten responses in carrier-primed animals (for example, ref. 10). Once these studies showed that carrier-primed T cells help hapten-primed B cells *in situ*, the matter of the anomalous response failures faded into the background and investigations shifted to exploring the mechanism of T-B interactions in adoptive transfer systems that avoid problems associated with *in situ* responses.

The conditions required to demonstrate carrier-specific help *in situ*, however, are of interest: before challenge with carrier and hapten-carrier conjugate, animals must be primed with the hapten (on an unrelated carrier)¹⁰. This suggests that the induction of hapten-specific suppression fails when cell populations that normally arise during hapten priming (that is, anti-hapten memory B cells and their idiosyncrasy-specific helper T cells) are present before the carrier/hapten-carrier exposure sequence. Which, if either, population prevents suppression induction is unclear; however, the participation of idiosyncrasy-specific help would be consistent with the idea that antibody responses are controlled by idiosyncrasy-specific "core regulatory circuits"¹¹ configured such that they tend to stabilize in either a suppression or a help mode (depending on whether more suppressor or more helper T cells are induced by the initial priming conditions).

Table 2 Hapten-carrier stimulation of anti-hapten memory B cells is normal in carrier-primed mice

Group	Memory B-cell donors form of antigenic stimulation		Adoptive IgG anti-DNP response	
	KLH	DNP-KLH	Units	Mean K_a ($\times 10^6$)
I	*	Alum	73	10
II	Alum	Alum	73	8
III	*	Aqueous	18	0.7
IV	Alum	Aqueous	50	8

Donors (BALB/c $\times$ SJL) from groups listed in Table 1 were killed 3 weeks after the first indicated stimulation with DNP-KLH. Splenic B cells were prepared by cytotoxic depletion of T cells using monoclonal anti-Thy-1.2 (30-H12, J. Ledbetter). Cells obtained from 10^7 spleen cells were co-transferred with 2×10^6 nylon-passed syngeneic T cells from KLH-primed donors (100 μ g on alum plus 10^9 *Bordetella pertussis* at least 6 weeks before transfer). Recipients (650 rad irradiated BALB/c) were stimulated with 1 μ g aqueous DNP-KLH (intravenous) at time of transfer and bled 1 week later for assay. Assay details are as described in Table 1 legend. IgG1b (IgG2a) response data are shown here. IgG1a (IgG2a) and IgG1 response data were similar. Responses in mice receiving nylon T (no donor B) were three units of very low affinity antibody. The proportion of IgD⁺ memory (also an index of memory population maturity³) was similar in groups I, II and IV and higher in group III, indicating less maturation (to IgD⁻) in mice primed with aqueous antigen. Anti-KLH memory was similar in groups I, II and IV and lower in group III.

*No KLH injection.

In any event, the dramatic reduction of the high affinity IgG anti-DNP response that we have shown to occur in KLH-primed mice subsequently exposed to DNP-KLH now brings hapten-specific regulatory processes into the arena of commonly studied heterogeneous antibody responses. Furthermore, it suggests an even closer functional interrelationship between carrier-specific and hapten (idiotype?)-specific regulation than previously supposed, as it indicates that carrier-specific priming contributes to the induction of hapten-specific suppression. This offers some entirely new perspectives on the mechanisms involved in response regulation and possibly on mechanisms of tolerance induction and prevention of autoantibody production.

These studies were supported by grants from the NIH (CA-04681, HD-01287, AI-08917).

Received 5 February; accepted 31 March 1980.

1. Mitchison, N. A. *Eur. J. Immun.* **1**, 10-17 (1971).
2. Rajewsky, K., Schirmacher, V., Nase, S. & Jerne, N. K. *J. exp. Med.* **129**, 1131-1143 (1969).
3. Herzenberg, L. A., Black, S. J., Tokuhisa, T. & Herzenberg, L. A. *J. exp. Med.* (in the press).
4. Hetzelberger, D. & Eichmann, E. *Eur. J. Immun.* **8**, 839-846 (1978).
5. Woodland, R. & Cantor, H. *Eur. J. Immun.* **8**, 600-606 (1978).
6. Fazekas de St. Groth, S. *Cold Spring Harb. Symp. quant. Biol.* **32**, 525-536 (1967).
7. Sarvas, H., Mäkelä, O., Toivanen, P. & Toivanen, A. *Scand. J. Immun.* **3**, 455-460 (1974).
8. Tada, T. in *Immunological Tolerance* (eds Katz, D. H. & Benacerraf, B.) 471-492 (Academic, New York, 1974).
9. Eardley, D. & Gershon, R. K. *J. exp. Med.* **142**, 524-529 (1975).
10. Katz, D., Paul, W. E., Goidl, E. A. & Benacerraf, B. *J. exp. Med.* **132**, 261-282 (1970).
11. Herzenberg, L. A., Black, S. J. & Herzenberg, L. A. *Eur. J. Immun.* **10**, 1-11 (1980).
12. Tsu, T. T. & Herzenberg, L. A. in *Selected Methods in Cellular Immunology* ch. 18, 373-397 (eds Mishell, B. B. & Shiigi, S. M.) (W. H. Freeman, San Francisco, 1980).

Lack of M-MuSV tumour regression associated with T lymphocyte tolerance

Luigi Chieco-Bianchi, Dino Collavo,
Paola Zanovell & Anita De Rossi

Laboratory of Oncology, University of Padova, Padova, Italy

Anthony J. S. Davies

Division of Biology, Chester Beatty Research Institute, Fulham Road, London SW3 6JB, UK

Moloney murine leukaemia virus (M-MuLV) and Moloney murine sarcoma virus (M-MuSV) are competent and replication defective forms, respectively, of a type C retrovirus which is oncogenic in some inbred mouse strains¹. M-MuLV induces lymphomas, mostly of T lymphocyte origin, following injection into newborn animals. M-MuSV together with M-MuLV, its natural helper, and in the absence of an appropriate immune response can elicit the formation of an unusual sarcoma which may grow and kill. This sarcoma is unusual in that, at least initially, it is a polyclonal entity within which continual recruitment of previously uninvolved mesenchymal cells occurs². Resistance to the induction of tumours by M-MuSV is thought to depend on activation of appropriate T lymphocytes. The main evidence for this is the finding that animals numerically deficient in T lymphocytes are highly susceptible to sarcoma induction³⁻⁵ and that normal mice and previously challenged animals can generate T lymphocytes specifically cytotoxic *in vitro* for tumour cells carrying viral antigens⁶. Animals injected with M-MuLV at birth are susceptible to M-MuSV challenge and incapable of generating cytotoxic T lymphocytes active against virus-infected cells *in vitro*^{7,8}. On the other hand, the M-MuLV

neonataly injected animals immunized as adults against allogeneic leukaemic cells are fully competent in generating alloreactive cytotoxic T lymphocytes⁹. T-cell deficient mice can in addition be reconstituted in their capacity to resist challenge by M-MuSV by implantation of syngeneic thymus grafts or injection of normal T lymphocytes^{4,5}. The exact role of T lymphocytes in the control of the acute phase of the infection with M-MuSV is not certain but it probably involves a cytotoxic capacity of T cells comparable with that seen *in vitro*. The role of antibody in bringing about resistance is not clear, as passive immunization *per se* is relatively ineffective in preventing tumour growth in T-cell deficient mice⁵, as was transfer of specifically primed B cells (our unpublished data). We present here evidence that neither thymuses nor peripheral lymphocytes removed from mice injected with M-MuLV at birth are capable of reconstituting T-cell deficient mice in such a manner as to promote resistance to challenge with M-MuSV.

CBA/Ca (CBA) male mice, 4 to 6 weeks old, were rendered numerically deficient in T cells by a sequence of adult thymectomy, 850 rad total-body irradiation and injection with syngeneic bone marrow cells, as detailed elsewhere⁵. The mice were transplanted with one thymus lobe under the kidney capsule or were given an intraperitoneal (i.p.) injection of lymph node cells in suspension⁵. Thymuses to be grafted and lymph node cells for injection were obtained from donor syngeneic mice which had been injected subcutaneously (s.c.), within 48 h after birth, with 0.05 ml of 0.1 gEq cell-free extracts of primary leukaemias induced by M-MuLV, passage A Gross-(G-) MuLV in BALB/c or CBA mice, respectively.

Table 1 Growth of M-MuSV tumours in T-cell deprived mice receiving thymus graft from syngeneic donors neonatally injected with MuLV

Group	Total no. of mice	No. of mice with tumour	No. of mice with regressed tumour	No. of mice dead with tumours
Deprived	35	33	1	32
Deprived + thy graft from normal donors	11	11	10	1
Deprived + thy graft from M-MuLV injected donors	22	21	1	20
Deprived + thy graft from passage A G-MuLV injected donors	5	4	4	0

Four- to six-day old mice were used as thymus donors; thymus graft was performed 5 days before M-MuSV injection in recipient mice.

Normal syngeneic mice of comparable age and sex served as alternative thymus or lymph node cell donors. Mice of the various groups were challenged intramuscularly (i.m.) in the thigh region with 0.05 ml of pooled M-MuSV extract, a virus dose which was equivalent to 5 to 8×10^4 focus-forming units (FFU) when titrated on 3T3/FL cells. The time sequence of the various operations is indicated in Tables 1 and 2.

Table 1 reports the evolution of tumours induced by M-MuSV in deprived mice grafted with a thymus lobe obtained from normal or M-MuLV-injected donors. Most of the deprived mice bearing M-MuSV tumours eventually died with progressive tumour growth, although only a few of those reconstituted with a normal thymus graft were killed. The protective effect conferred by thymus grafting was not seen when the mice were transplanted with a thymus lobe from donors that had been neonatally injected with M-MuLV. All

these mice, with one exception, developed tumours which did not regress. Deprived mice were also transplanted with a thymus lobe derived from donors neonatally injected with passage A G-MuLV, known to induce type-specific tumour associated antigens distinct from those determined by M-MuLV. Protection was achieved in this group, suggesting that unresponsiveness in mice implanted with thymuses from 'tolerized' mice was specific. (Note that, at autopsy, the grafted thymic tissue was always detected macroscopically and appeared histologically normal.)

Table 2 Growth of M-MuSV tumours in T-cell deprived mice receiving lymph node cells from donors neonatally injected with MuLV

Group	Total no. of mice	No. of mice with tumour	No. of mice with regressed tumour	No. of mice dead with tumours
Deprived	15	15	0	15
Deprived + LN cells from normal donors	10	6	4	2
Deprived + LN cells from M-MuLV injected donors	16	14	0	14
Deprived + LN cells from passage A G-MuLV injected donors	7	5	3	2

Deprived mice, 10 days before M-MuSV inoculation, were given intraperitoneally 10×10^6 lymph node cells from 6 to 8-week old donors.

As shown in Table 2, 10×10^6 lymph node cells, from normal or passage A G-MuLV neonatally injected mice, transferred i.p. 10 days before M-MuSV inoculation were effective in preventing tumour development as well as in reducing mortality in the recipients. No protection was afforded by lymph node cells from M-MuLV neonatally infected donors since all the 14 mice developing tumours eventually died with extensive neoplastic involvement of thigh and pelvic tissues.

The diversity in tumour behaviour cannot easily be imputed to differences in the degree of T-cell reconstitution because both passage A G-MuLV neonatally injected, M-MuSV tumour regressors and M-MuLV neonatally injected mice bearing progressing tumours possessed comparable T cell levels—about 20–25% of the total peripheral blood lymphocyte population. These values (assessed 5 weeks after lymph node cell transfer, in a cytotoxic assay with anti Thy 1.2 serum and guinea pig serum as a complement source) were comparable with those observed in deprived mice reconstituted with normal lymph node cells. Less than 5% of T cells were found in the blood of tumour bearing T-cell deficient mice which had received no reconstituting lymphocytes.

In our experiments the latency period for tumours after the challenging injection of M-MuSV was about 16 days in all groups of mice except those which received lymph node cells from neonatally injected mice, in which the latency period was about 30 days, with death within 2 months. No explanation can be offered for this finding at present.

Tests were carried out for the presence of virus in some of the lymph node donor animals. M-MuLV titre was determined on tail tissue extracts using the SC-1/XC cell plaque assay as previously reported⁹. Mice injected at birth with M-MuLV had a titre of 10^4 plaque-forming units (PFU) per ml of 2% tail extract, whereas normal control mice showed negligible M-MuLV production ($<10^1$ PFU). Further, aliquots of the

lymph node cell suspensions used to reconstitute T-cell deprived mice were tested for M-MuLV production by co-cultivation with SC-1 cells and after 4 days the cultures were UV irradiated and tested for XC plaques as detailed elsewhere¹⁰. Lymph node cell suspensions from neonatally M-MuLV injected mice had a titre of $1-5 \times 10^4$ PFU per 10×10^6 lymph node cells, whereas lymph node cell suspensions from control mice showed $<10^1$ PFU per 10×10^6 lymph node cells.

The experimental results obtained are in line with the general phenomenon of immunological tolerance which can follow introduction of foreign antigens at an early stage of development. The indications are also that the tolerance observed is along the T-cell axis.

In line with this, it has been shown⁸ that mice neonatally injected with M-MuLV produce antibodies specific for the virus components despite their incapacity to generate cytotoxic T cells against virally infected cells. However, it cannot be assumed that the tolerance involved is entirely T-cell rather than B-lymphocyte orientated. This conclusion would require a more rigorous assessment of the quantities and quality of anti-viral antibodies produced, particularly in view of the possibility that type C retroviruses other than the injected M-MuLV (that is, endogenous eco- and xeno-tropic, as well as 'recombinant' new viruses) may be expressed in the tolerized hosts.

It is intriguing that the viral yield was high in the tolerized mice and in the lymphocytes transferred from them. Furthermore, our preliminary experiments indicate that thymus, spleen and lymph node cells a few days after neonatal injection of animals with M-MuLV already possess surface antigenic components which are recognized as targets by M-MuSV immune spleen cells in a short-term incubation ⁵¹Cr-release assay. The lymphoid cells from M-MuLV infected mice, when used as stimulators, are also able to elicit the generation of cytotoxic T cells *in vitro* (data not shown). Thus it could be argued that the tolerized cells were unable to distinguish, as foreign, virus-associated antigens that they lived with and accepted as self. Note also the additional possibility that the thymic epithelium of thymus grafts from tolerized mice had the capacity to imprint on those lymphocytes that it processed, subsequent to tolerogenesis, the ability to recognize viral antigens as self. To determine whether such a process is distinguishable from infection would require further study. Finally, we have obtained no evidence to show that suppressor T cells exert an active role in the tolerance phenomenon (manuscript in preparation): such a role has been suggested to explain the results of some experiments in which rats were injected with G-MuLV¹¹.

This work was supported by grants from Consiglio Nazionale delle Ricerche (P. F. Controllo Crescita Neoplastica N. 78.02816.96 and 78.02819.96), and Associazione Italiana per la Ricerca sul Cancro.

Received 2 October 1979; accepted 24 March 1980.

- Levy, J. P. & Leclerc, J. C. *Adv. Cancer Res.* **24**, 1–66 (1977).
- Chieco-Bianchi, L. & Collavo, D. in *Scientific Foundations of Oncology* (eds Symington, T. & Carter, R. L.) 388–393 (Heinemann, London 1976).
- Stutman, O. in *The Nude Mouse in Experimental and Clinical Research* (eds Fogg, J. & Giovarelli, B.) 411–435 (Academic, New York, 1978).
- Collavo, D., Colombatti, A., Biasi, G., Chieco-Bianchi, L. & Davies, A. J. S. *Nature* **249**, 169–170 (1974).
- Collavo, D., Colombatti, A., Biasi, G., Chieco-Bianchi, L. & Davies, A. J. S. *J. natn. Cancer Inst.* **56**, 603–608 (1976).
- Collavo, D., Parenti, A., Biasi, G., Chieco-Bianchi, L. & Colombatti, A. *J. natn. Cancer Inst.* **61**, 885–890 (1978).
- Chieco-Bianchi, L. et al. in *Tumour-associated Antigens and their Specific Immune Response* (eds Spreafico, F. & Arnon, R.) 71–84 (Academic, New York, 1979).
- Chieco-Bianchi, L., Sando, F., Aoki, T. & Barrera, O. L. *J. natn. Cancer Inst.* **52**, 1345–1350 (1974).
- Colombatti, A., De Rossi, A., Taylor, B. A., Chieco-Bianchi, L. & Meier, H. *Int. J. Cancer* **21**, 179–185 (1978).
- Colombatti, A., De Rossi, A., Hilken, J., Collavo, D. & Chieco-Bianchi, L. *J. natn. Cancer Inst.* **62**, 1451–1457 (1979).
- Myburgh, J. A. & Mitchison, N. A. *Transplantation* **22**, 236–244 (1976).

Corpus allatum is release site for insect prothoracicotropic hormone

N. Agui

Department of Entomology, The National Institute of Health, Kamiosaki, Shenagawa-ku, Tokyo 141, Japan

W. E. Bollenbacher, N. A. Granger & L. I. Gilbert

Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201

The primary effector of insect postembryonic development is a peptide neurohormone, the prothoracicotropic hormone (PTTH)^{1,2}. PTTH is the product of a single neurosecretory cell (NSC) in each hemisphere of the protocerebrum of the tobacco hornworm, *Manduca sexta*³. Its neurohaemal organ, however, has not previously been identified. Determination of the site of release would complete the identification of the neuroendocrine axis of PTTH and establish the boundaries of an intracellular system within which factors controlling the synthesis, transport, storage and release of an identified neurohormone could be investigated. The neurohaemal organ for PTTH has been assumed to be the corpus cardiacum (CC), a structure located between the brain and corpus allatum (CA)^{1,2}, but alternative sites of release have been suggested, including the CA⁴⁻⁷ and the brain itself^{8,9}. In this study, a novel *in vitro* assay for PTTH^{10,11} has been used to identify the CA as the neurohaemal organ for PTTH in *Manduca*.

Establishment of the neurohaemal organ for PTTH required the identification of the cerebral NSC responsible for its synthesis and their axonal distribution, as well as a titre of PTTH in the brain and retrocerebral complex (CC plus CA) during metamorphosis. The PTTH NSC in freshly ecdysed pupae of *Manduca* have recently been identified^{3,11} and a previous cobalt backfill study of NSC in the pupal brain demonstrated that the axons of the PTTH NSC terminate in the CA¹². These data suggest that the CA may be the site of release, but do not preclude the CC as the neurohaemal site (because of the limitations of the backfill technique, such as interneuronal migration¹³). However, in conjunction with the cell localization and backfill data, a titre of PTTH in the CC and CA during larval-pupal metamorphosis could directly establish the neurohaemal site for this hormone.

To establish the CA as the neurohaemal site, it had to be demonstrated that the CA contained most of the PTTH activity in the retrocerebral complex at critical stages of development and that the levels approximated those for the brain. Since the PTTH NSC axons destined to terminate in the CA must traverse the CC, the CC would presumably possess only a low level of PTTH activity. To quantify PTTH activity, a dose-response protocol for *in vitro* activation of the prothoracic glands (PG) by the neurohormone was used^{3,10,11}. Before assaying PTTH, it was necessary to prove that the activity being assayed was due to PTTH. This was accomplished by demonstrating the heat stability of the PTTH activity in the CC and CA and a dose-related response for activation of the PG by supernatants of homogenates of the brain, CC and CA from day 0-1 pupae (Fig. 1). In addition, the sum of the reciprocals of the A_{50} tissue equivalents (see Fig. 1 legend) for each component closely approximated the reciprocal of the A_{50} tissue equivalents for the brain plus retrocerebral complex, thus demonstrating that the PTTH activity was quantifiable. The possibility of juvenile hormone (JH) involvement in PG activation² by CA homogenates was eliminated by showing that endogenous

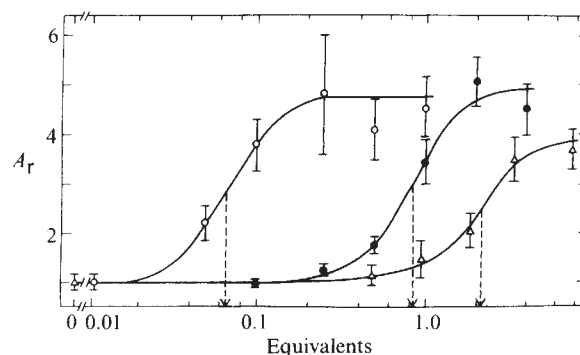


Fig. 1 Dose response of PG activation by brain (○), CA (●) and CC (△) from day 0-1 *Manduca* pupae. The PTTH assays were performed on supernatants of heat-treated (100 °C, 2 min) tissue extracts¹⁰. The abscissa represents the tissue equivalents assayed with one equivalent equal to 1 brain, 2 CA (1 pair) or 2 CC (1 pair). Activation is expressed as an activation ratio (A_r) obtained by dividing the amount of ecdysone synthesized by the experimental gland by that synthesized by the control gland. Ecdysone was quantified by radioimmunoassay. The total PTTH activity present in the brain plus retrocerebral complex could be quantified by summing the reciprocals of the equivalents for each tissue (brain, CC, CA) necessary to elicit half maximal activation (A_{50}) of ecdysone synthesis by the PG^{3,11}. Thus, the reciprocals of the A_{50} values (dashed vertical lines) for the brain (0.066), CA (0.84) and CC (2.15) were 15.1, 1.2 and 0.5, respectively. Their sum equals 16.8, which is 107% that of the reciprocal value obtained with the A_{50} tissue equivalent for the brain plus retrocerebral complex assayed together (15.7). Each datum point represents the average $\pm$ s.e.m. of three separate assays, each run in triplicate.

levels of JH in the CA are very low¹⁴ and that the supernatant of a CA homogenate following a hexane partition to remove JH elicited the same dose response of PG activation as an untreated supernatant.

The PTTH titre of the CC and CA was then determined for the last larval instar and early pupal development (Fig. 2). The data revealed that PTTH activity in the CA was always significantly greater than that in the CC. Early in the last instar (day 1-3), an increase in activity was observed for the CA in contrast to a decrease in CC activity. An increase in PTTH activity in the CA at this time would be expected if this gland were the neurohaemal organ since presumably PTTH is released at ~day 4 (ref. 15) to elicit the small ecdysteroid peak¹⁶ responsible for a change in commitment from larval to pupal development¹⁷. The slight decrease in activity in the CA between days 3 and 5 may reflect this release. Although dramatic changes in the level of PTTH activity in the CA did not occur until pupation, there was another slight increase between days 5 and 7, possibly in preparation for PG activation at ~day 8 (ref. 15); the resulting second ecdysteroid peak elicits apolysis and pupal ecdysis^{15,16}. Again, a very slight decrease in CA activity was noted between days 7 and 9, possibly indicating that release had occurred. A large decrease in PTTH activity occurred just at pupation, the endocrinological significance of which is not known. During the first 24 h after the pupal moult, the PTTH activity in the CA reached its maximum titre, presumably in preparation for release at approximately day 2 of pupal life (L. M. Riddiford, personal communication). The slight decrease in CA activity expected after PTTH release was again observed (day 1-3). An increase in PTTH activity also occurred in the CC during pharate pupal development and again at pupation, probably due to a higher level of this hormone in the traversing NSC axons.

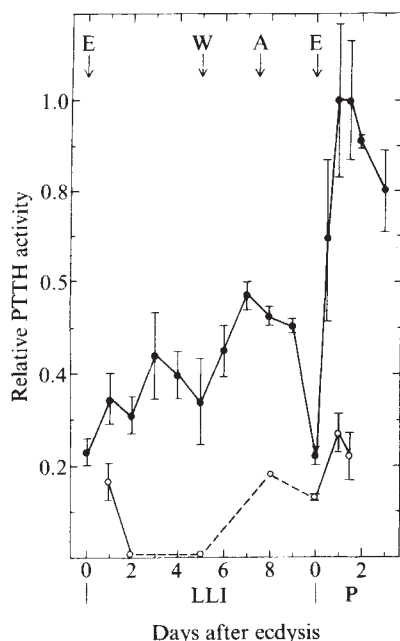


Fig. 2 Titre of PTTH activity in the CA (●) and CC (○) during the last larval instar (LLI) and early pupal stage (P) of *Manduca*. PTTH activity was quantified using a dose-response protocol and is expressed relative to the hormone activity of one pair of day 1 pupal CA. E, W and A signify the times of ecdysis, wandering and apolysis, respectively. Each datum point represents an average $\pm$ s.e.m. of three to four separate assays, each run in triplicate (see Fig. 1). The s.e.m. values for CC at days 2 and 5 were smaller than the data points, while the day 8 value represents one determination run in triplicate. Dashed lines signify that daily determinations were not made during that period.

These data reveal that during larval-pupal development, the CA contains far greater PTTH activity than the CC. In addition, since hormonal activity in a neurohaemal organ would probably reflect activity at the site of synthesis, it is significant that changes in the titre of PTTH in the CA are similar to those noted for the brain of *Manduca* during the same developmental period (unpublished). Thus, based on the identification of the PTTH NSC in the brain, the axonal distribution pattern of these NSC, and the titre of PTTH activity in the CA during larval-pupal development, it is concluded that the CA is the neurohaemal organ for PTTH in *Manduca sexta*.

We thank Nancy Grousnick for clerical assistance. This research was supported by grants from NIH and NSF.

Presence of a low molecular weight endogenous inhibitor on ^3H -muscimol binding in synaptic membranes

Yukio Yoneda & Kinya Kuriyama

Department of Pharmacology, Kyoto Prefectural University of Medicine, Kamikyo-Ku, Kyoto 602, Japan

The specific binding of ^3H -muscimol to synaptic membrane preparations obtained from the rat brain has been thought to reflect the association of γ -aminobutyric acid (GABA), a potential candidate as an inhibitory neurotransmitter in the mammalian central nervous system (CNS), with its synaptic receptors^{1,2}. Treatment of synaptic membranes with Triton X-100 significantly increases the specific binding of ^3H -muscimol². Several reports also indicate the presence of endogenous substances, such as GABA³, acidic protein⁴ and phosphatidylethanolamine⁵, which inhibit Na-independent binding of ^3H -GABA in the synaptic membranous fractions from the rat brain. We report here that in the supernatant obtained from Triton-treated synaptic membranes there exists a new type of endogenous inhibitor of ^3H -muscimol binding which is apparently different from the inhibitory substances described previously³⁻⁵. The new inhibitor has a low molecular weight (MW) and probably originated from neurones rather than glial cells. We have termed this endogenous inhibitor the GABA receptor binding inhibitory factor (GRIF).

Treatment of crude synaptic membrane fractions obtained from the rat brain with Triton X-100 significantly increased the specific binding of ^3H -muscimol². One explanation for this increase in binding is that an endogenous binding inhibitory substance may be present in synaptic membranes and that this inhibitor is removed by the Triton treatment. In fact, it has been reported that an endogenous inhibitor of the Na-independent binding of ^3H -GABA is present in the particulate³ as well as the crude synaptic membrane fractions^{4,5} of the rat brain.

The supernatant (Sup T) obtained from Triton-treated synaptic membranes, when added to the assay mixture for ^3H -muscimol binding, produced a drastic reduction of specific ^3H -muscimol binding to Triton-treated as well as to untreated normal membranes (Fig. 1a). Addition of a large amount (0.9 ml) of Sup T almost completely inhibited the specific binding without affecting nonspecific binding. These results strongly suggest that an endogenous inhibitor of ^3H -muscimol binding is present in synaptic membrane fractions and is removed from the membranes by the Triton treatment.

It has been shown that synaptic membrane preparations contain low- and high-affinity binding sites for ^3H -muscimol and the former site is easily converted into the latter form by Triton X-100 treatment². This phenomenon has also been confirmed in the present study. Therefore, we examined the effect of Sup T on high-affinity ^3H -muscimol binding to Triton-treated synaptic membranes. Scatchard analysis of the data from experiments on Sup T-induced inhibition of high-affinity ^3H -muscimol binding to Triton-treated membranes revealed that the apparent affinity (K_d) of ^3H -muscimol for GABA receptor binding sites is reduced, whereas the number of maximal binding sites (B_{max}) is not affected by the addition of Sup T (Fig. 1b). These results coupled with our recent findings that Triton treatment makes the high-affinity binding sites more sensitive to alterations of external pH and temperature (unpublished data), suggest that the inhibitor in Sup T may exhibit its action by reducing the affinity and inducing conformational changes in the binding sites.

Received 5 November 1979; accepted 8 April 1980.

- Wigglesworth, V. B. *Insect Hormones* (Freeman, San Francisco, 1970).
- Gilbert, L. I. & King, D. S. in *The Physiology of Insecta I* (ed. Rockstein, M.) 249-370 (Academic, New York, 1973).
- Agui, N., Granger, N. A., Gilbert, L. I. & Bollenbacher, W. E. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5694-5698 (1979).
- Ichikawa, M. & Nishitsutsuji-Uwo, J. *Biol. Bull. Woods Hole* **116**, 88-94 (1959).
- Ishizaki, H. *Dev. Growth, Diff.* **11**, 1-7 (1969).
- Gibbs, D. & Riddiford, L. M. *J. exp. Biol.* **66**, 255-266 (1977).
- Morohoshi, S. & Shimada, J. *Proc. Japan Acad.* **51**, 744-748 (1975).
- Nishitsutsuji-Uwo, J. *Z. Zellforsch.* **54**, 613-630 (1961).
- Takeda, N. *Appl. Ent. Zool.* **11**, 143-153 (1976).
- Bollenbacher, W. E., Agui, N., Granger, N. & Gilbert, L. I. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5148-5152 (1979).
- Bollenbacher, W. E., Agui, N., Granger, N. & Gilbert, L. I. in *Invertebrate Systems In Vitro* (eds Kurstak, E., Maramorosch, K. & Dübendorfer, A.) (Elsevier, Amsterdam, in the press).
- Nijhout, H. F. *Int. J. Insect Morph. Embryol.* **4**, 529-538 (1975).
- Strausfeld, N. F. & Obermayer, M. *J. comp. Physiol.* **110**, 1-12 (1976).
- Granger, N. A. *et al. Molec. cell. Endocr.* **16**, 1-17 (1979).
- Truman, J. W. & Riddiford, L. M. *J. exp. Biol.* **60**, 371-382 (1974).
- Bollenbacher, W. E., Vedeckis, W. V., Gilbert, L. I. & O'Connor, J. D. *Dev. Biol.* **44**, 46-53 (1975).
- Riddiford, L. M. *Nature* **259**, 115-117 (1976).

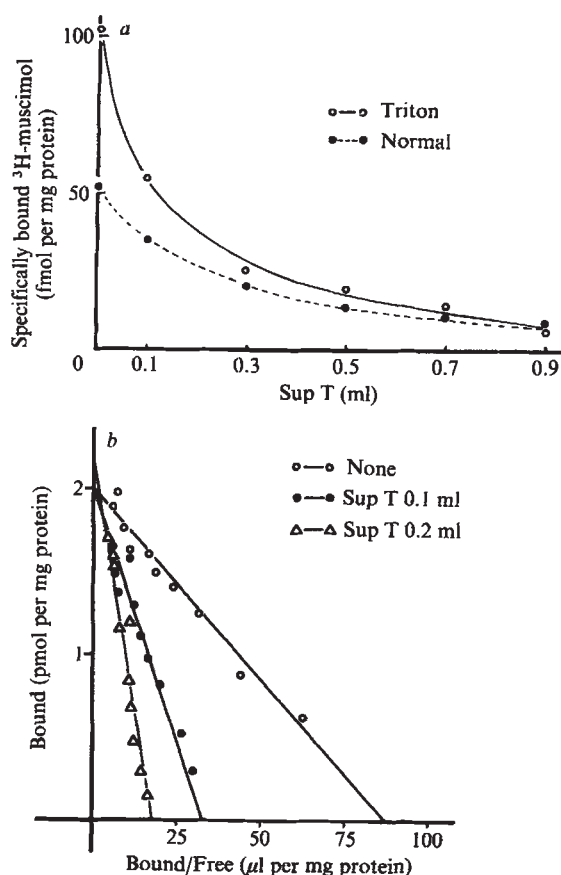


Fig. 1 Effect of *in vitro* addition of Sup T on specific ³H-muscimol binding. *a*, Inhibition with increasing concentration of Sup T; *b*, Scatchard analysis of Sup T-induced inhibition of the binding to Triton-treated synaptic membranes. Crude synaptic membrane fractions were obtained from the whole brain (including cerebellum) of male Wistar rats (200–250 g) as previously reported¹³. After repeating the washing procedures three times using 50 ml of 50 mM Tris-citrate buffer (pH 7.1) to remove endogenous GABA, the pellet finally obtained was frozen at -20°C for 1–7 days. These frozen pellets were thawed and washed once with 50 ml of Tris-citrate buffer before each use. For preparing the Triton-treated membranes, suspensions of the frozen-thawed pellets were preincubated with 0.01% of Triton X-100 at 25°C for 30 min instead of washing by buffer. The preincubation with Triton X-100 was terminated by the centrifugation at 48,000 g for 20 min. The supernatant (Sup T) obtained from the Triton-treated membranes was directly added to the assay system for ³H-muscimol binding as needed. The assay system for ³H-muscimol binding was as follows. The membranous suspensions treated or not treated with Triton X-100 were incubated with 1 nM of ³H-muscimol ([Me-³H]3-hydroxy-5-aminomethyl isoxazole, 12.9 Ci mmol⁻¹, NEN) in 1 ml of 50 mM Tris-citrate buffer (pH 7.1) at 2°C for 30 min. The incubation was terminated by the addition of 2 ml of ice-cold buffer and subsequent filtration with membrane filter (pore size: 0.45 μm, Toyo Roshi) under vacuum¹⁰. After washing the filter twice with 2 ml of ice-cold buffer, the radioactivity trapped on the filter was measured as described previously¹⁰. The radioactivity found in the presence of 1 mM nonradioactive GABA was considered to be due to nonspecifically bound ³H-muscimol and subtracted from each experimental value to obtain the specific binding of ³H-muscimol. The specific binding of ³H-muscimol was saturable with increasing concentration of ³H-muscimol and indicated the presence of two different types of binding site: a high-affinity site with a K_d of 4.7 nM and a low-affinity site having a K_d of 90 nM. The maximal binding to the high- and low-affinity sites is 0.6 and 1.7 pmol per mg protein, respectively. These values are essentially in agreement with the previously reported values². The protein concentration usually used was 400 μg protein per assay. Protein content in Sup T was 150 ± 30.8 μg protein per ml. For Scatchard analysis (*b*), the membranous preparations were incubated with 10 nM of ³H-muscimol in the absence and presence of various concentrations of nonradioactive muscimol which was synthesized in our laboratory according to the method of Nakamura¹⁴. Triton treatment only: $B_{\text{max}} = 2.0$ pmol per mg protein, $K_d = 22$ nM; Sup T 0.1 ml added: $B_{\text{max}} = 2.0$ pmol per mg protein, $K_d = 52$ nM; Sup T 0.2 ml added: $B_{\text{max}} = 2.2$ pmol per mg protein, $K_d = 163$ nM. Each determination was always carried out in triplicate and each varied less than 10%. Values given represent the mean of at least two separate experiments. Protein was determined by the method of Lowry *et al.*¹⁵.

Column chromatographic analysis of Sup T using Dowex 50W×8 resin revealed that this inhibitor co-eluted with ³H-GABA (Fig. 2a). The peak of inhibitory activity of Sup T was, however, completely separated from that of ³H-GABA when the Sup T was applied to a Sephadex G-10 column (16×180 mm) (Fig. 2b). These results clearly indicate that the inhibitory activity of Sup T on the binding is not derived from GABA itself. As *in vitro* addition of Triton X-100 did not cause significant inhibition as Sup T (Table 1; maximal concentration of Triton X-100 derived from Sup T is calculated to be less than 0.005%), we conclude that the endogenous inhibitor, which we term the GABA receptor binding inhibitory factor (GRIF), is indeed present in the synaptic membrane fractions.

The inhibitory action of GRIF was greatly attenuated following the dialysis of Sup T, whereas boiling and enzymatic treatments of Sup T with trypsin, collagenase, peroxidase and phospholipase C had no significant effect (Table 1). GABA-induced inhibition of ³H-muscimol binding was diminished by the addition of fluorecamine, but the inhibition by GRIF was not affected by this primary amine reactive reagent (Table 1). These results suggest that GRIF may be a thermostable substance with a low MW and that no primary amine structure is involved in its activity. In fact, the apparent MW of GRIF was estimated to be less than 500 according to the filtration experiments using the Amicon Diaflo membrane UM05 (Table 1).

Table 1 Some characteristics of GRIF in Sup T

Addition	Specifically bound ³ H-muscimol (fmol per mg protein)
None	104.3 ± 5.8 (10)
Triton X-100	
0.01 %	94.0 ± 2.4 (5)
0.05 %	70.3 ± 4.5 (5)
Sup T (0.5 ml)	
Untreated	31.5 ± 4.3 (5)
Dialysis (2 h)	82.7 ± 3.6 (5)
Ether extraction	27.2 ± 2.0 (5)
Boiling (95 $^{\circ}\text{C}$, 10 min)	30.9 ± 4.7 (5)
Trypsin (500 μg ml ⁻¹)	33.7 ± 5.5 (5)
Collagenase (500 μg ml ⁻¹)	32.8 ± 3.0 (5)
Peroxidase (500 μg ml ⁻¹)	32.4 ± 4.7 (5)
Phospholipase C (500 μU ml ⁻¹)	29.7 ± 2.9 (5)
Sup T (0.1 ml)	59.6 ± 2.1 (5)
Sup T (0.1 ml) + fluorecamine	61.2 ± 3.8 (5)
Fraction 14 (GRIF) (0.1 ml)	23.6 ± 2.7 (4)
Fraction 14 (0.1 ml) + fluorecamine	22.1 ± 2.5 (4)
GABA 10^{-7} M	40.2 ± 3.4 (5)
GABA 10^{-7} M + fluorecamine	72.9 ± 1.7 (5)
GABA 10^{-6} M	5.3 ± 1.2 (5)
GABA 10^{-6} M + fluorecamine	13.7 ± 2.2 (5)
GABA 10^{-5} M	0.5 ± 0.1 (5)
GABA 10^{-5} M + fluorecamine	3.8 ± 0.6 (5)
Filtration by Diaflo membrane UM05	
Filtrate	
0.1 ml	33.6 ± 1.2 (4)
0.5 ml	13.2 ± 0.9 (4)
Residue	
0.1 ml	89.3 ± 6.9 (4)
0.5 ml	70.5 ± 12.0 (4)

Treatments: Dialysis: 10 ml Sup T was dialysed against 300 ml of H₂O at 4°C for 2 h. Ether extraction: 5 ml Sup T was added to 20 ml ether and the mixture was vigorously shaken for 10 min. Following the centrifugation of this mixture at 3,000 g for 10 min, the upper layer thus obtained was removed by suction. These procedures were repeated four times. Enzyme treatments: following the incubation of Sup T with trypsin, collagenase, peroxidase or phospholipase C at 37°C for 2 h, the reaction was terminated by boiling the incubation mixture for 10 min. Fluorecamine reaction: 50 μl of 0.1% fluorecamine (w/v, in acetone) was rapidly added with stirring¹² to 1 ml GABA solution, Sup T or fraction 14 (see Fig. 2a) obtained following cation exchange chromatography. After taking away the acetone by a N₂-gas stream, each sample was subjected to the binding assay. Filtration experiments: 5 ml of concentrated Sup T was filtrated on the Amicon Diaflo membrane UM05 under N₂-gas pressure (5 kg cm⁻²). The solution (0.6 ml) remaining on the filter was diluted by H₂O into 5 ml. The diluted residue and the filtrate were subjected to the binding assay, respectively. Each value represents the mean $\pm$ s.e.m. obtained from various numbers of separate experiments as indicated in parentheses.

To determine whether or not GRIF indeed originates from neurones, we examined the effect of selective neuronal degeneration by kainic acid^{6,7} on the endogenous level of GRIF. Unilateral microinjection of kainic acid ($2\mu\text{g}\mu\text{l}^{-1}$) into the corpus striatum produced a significant degeneration of neurones at the injected side without affecting the non-injected side, as described elsewhere⁸. The inhibitory activity of Sup T obtained from the injected side of the corpus striatum is significantly lower than that from the non-injected side (Fig. 3a, b). As the neuronal degeneration induced by kainic acid significantly diminished the level of GRIF, it is reasonable to consider that GRIF may be present in neurones rather than in other CNS components such as glial cells.

Thus, we have demonstrated that in crude synaptic membrane fractions from the rat brain there is an endogenous inhibitor of GABA receptor binding (GRIF) which has a relatively low MW. GRIF is apparently different from the protein type of inhibitor of GABA receptor binding reported previously^{4,9}. The protein type of inhibitor showed an anionic property⁴, whereas GRIF was adsorbed to the cation exchange resin (Fig. 2a). The interrelationship between the protein type of inhibitor and GRIF, however, remains to be elucidated. It is conceivable that GRIF is one of the main active constituents of the protein type of inhibitor.

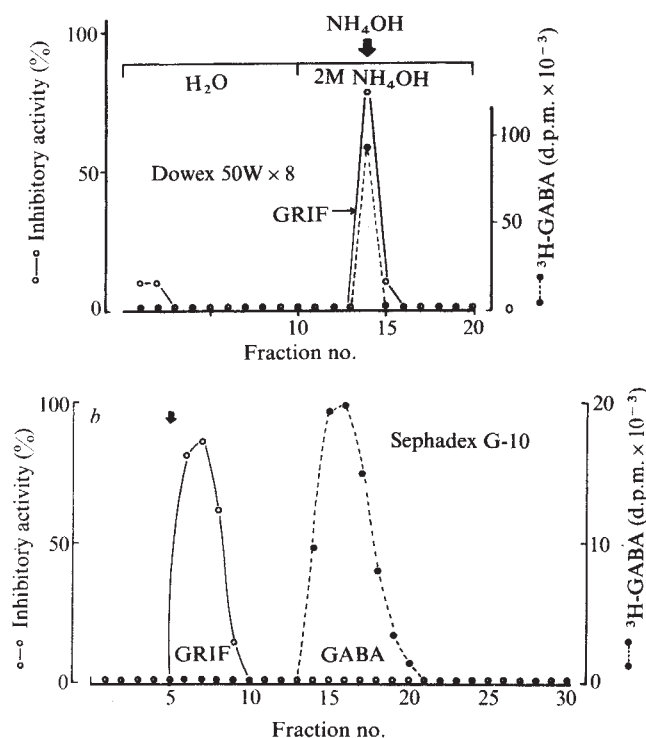


Fig. 2 Elution profiles of GRIF and ^3H -GABA. *a*, Dowex 50W x 8, 200–400 mesh, H^+ form; *b*, Sephadex G-10. Sup T was obtained as described in Fig. 1 legend and heated at 95°C for 10 min. Following the centrifugation of this boiled Sup T at $10,000g$ for 20 min, the supernatant was evaporated to dryness using a rotary evaporator at 40°C . The resultant residue was suspended with H_2O and the suspension was applied to the glass column packed with cation exchange resin Dowex 50W x 8 (200–400 mesh, H^+ form, $6 \times 120\text{mm}$). The column was eluted with H_2O and subsequently with $2\text{M NH}_4\text{OH}$. The eluate (2ml) was collected successively and evaporated to dryness to remove eluted NH_4OH . After suspending the residue with a similar volume (2ml) of H_2O , 0.2-ml portions of each fraction were added to the assay mixture for ^3H -muscimol binding to examine the effect on the binding. The activity of GRIF was eluted in consistency with the elution of NH_4OH . ^3H -GABA ($0.5\mu\text{Ci}$; 34.5Ci mmol^{-1} , NEN) was also applied to the column and similarly eluted. For experiments on Sephadex G-10 (*b*), the supernatant obtained from boiled Sup T was evaporated to dryness and suspended with 0.3ml of H_2O . The suspension was applied to the Sephadex G-10 column ($16 \times 180\text{mm}$) and the column was eluted with H_2O . The eluate (3ml) was collected successively and 0.1-ml portions of each fraction were subjected to the binding assay.

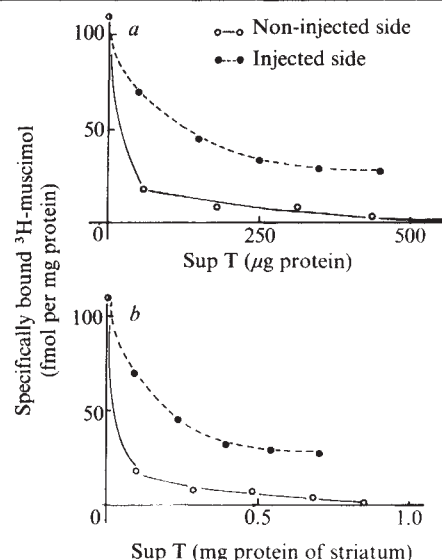


Fig. 3 Effect of neuronal degeneration by kainic acid on GRIF in Sup T. Animals were fixed with a stereotaxic apparatus under pentobarbital anaesthesia (50mg per kg , intraperitoneally) and kainic acid ($2\mu\text{g}\mu\text{l}^{-1}$) dissolved in the artificial cerebrospinal fluid was injected slowly (over 3 min) into the left corpus striatum using a Hamilton microsyringe. The needle was carefully removed 3 min after the end of injection. The stereotaxic coordinates were made according to Pellegrino and Cushman¹⁵ (A: 7.8, L: 3.0, V: 4.5 mm) and the exact location of the injected site was confirmed histologically. Seven days after the injection, animals were killed by decapitation and the corpus striatum was dissected out on a chilled glass. Samples from each unilateral side (injected side and non-injected side, respectively) obtained from 30 animals were combined and frozen at -20°C for 1–7 days. These frozen samples were sonicated in 50ml of ice-cold H_2O with a Polytron (setting no. 6 for 30 s) and the homogenates were centrifuged at $48,000g$ for 20 min. The resultant precipitates were resuspended in 50ml of 50mM Tris-citrate buffer ($\text{pH } 7.1$) and recentrifuged in the same conditions. After repeating these washing procedures twice to remove endogenous GABA, the pellets obtained were suspended in H_2O using a polytron. The suspensions obtained from each unilateral side of the corpus striatum were preincubated with 0.05% of Triton X-100 at 25°C for 30 min. Following termination of the preincubation by centrifuging at $48,000g$ for 20 min, the supernatant thus obtained (Sup T) was subjected to the binding assay. In these experimental conditions, there were significant reductions ($50\text{--}60\%$) in GABA content, activities of L-glutamic acid decarboxylase and GABA transaminase succinic semialdehyde dehydrogenase and the uptake and release of ^3H -GABA in the corpus striatum⁸. Similar tissue masses were dissected out from injected and non-injected sides of the corpus striatum, respectively ($31.4 \pm 2.1\text{mg}$ for the injected corpus striatum and $31.9 \pm 1.5\text{mg}$ for the non-injected corpus striatum). GABA contents in the final membranous preparations obtained from the injected and non-injected sides of the corpus striatum were 218 ± 53 and $276 \pm 88\text{pmol GABA per mg protein}$, respectively. It was also found that the specific binding of ^3H -muscimol is significantly increased at the injected side of the corpus striatum over that of non-injected side (68.9 ± 6.9 compared with $35.6 \pm 5.1\text{fmol per mg protein}$). Each value given represents the mean obtained from two separate experiments. *a*, Inhibitory activity is expressed as a function of the protein content in Sup T. *b*, Inhibitory activity is expressed as a function of the protein content in original striatal particulate preparation.

Recently, it has also been reported that the incubation of Triton-treated synaptic membrane with phosphatidylethanolamine, a phospholipid, significantly reduces the specific binding of ^3H -GABA without affecting the non-specific binding⁵. The concentration of the phospholipid required to induce half-maximal inhibition of the binding, however, seems to be relatively high (0.1mM) as compared with the small amount of Sup T required to induce half-maximal inhibition of the ^3H -muscimol binding. In addition, there are some property differences between phosphatidylethanolamine and GRIF such as solubility in ether, permeability to Diaflo membrane UM05 and participation of primary amine structure in its inhibitory activity. Considering these facts with the finding that GRIF completely inhibits the specific binding to untreated normal membrane as well as Triton-treated membrane, it is unlikely that GRIF is phosphatidylethanolamine itself.

We have also demonstrated that neuronal degeneration of the left striatum by kainic acid injection significantly increases the specific binding of ^3H -muscimol at the injection side and that this increase seems to result from the decrease of the endogenous level of GRIF¹⁰. Considering these previous findings with the results obtained in this study, it seems reasonable to conclude that the denervation supersensitivity phenomenon at the GABA receptor occurs as the result of removal of the endogenous inhibitor of receptor binding as suggested by Andrews and Johnston¹¹. Although we consider that GRIF may originate from neurones rather than glial cells, the exact location of this inhibitor is unclear. If GRIF is present in presynaptic terminals, it is possible that GRIF is released from presynaptic nerve endings together with GABA during neuronal excitation and modulates synaptic transmission at GABAergic synapses. Studies on biochemical characteristics and the exact physiological roles of GRIF are under way in our laboratory.

We thank Mr Etsuo Kurihara for providing us with kainic acid injected animals. This work was supported in part by a Grant-in-Aid for Scientific Research (nos 487047 and 421331) from the Ministry of Education, Science and Culture, Japan.

Received 19 February; accepted 17 April 1980.

- Snodgrass, S. R. *Nature* **273**, 392-394 (1978).
- Beaumont, K., Chilton, W., Yamamura, H. I. & Enna, S. J. *Brain Res.* **148**, 153-162 (1978).
- Greenlee, D. V., Van Ness, P. G. & Olsen, R. W. *Life Sci.* **22**, 1653-1662 (1978).
- Toffano, G., Guidotti, A. & Costa, E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4024-4028 (1978).
- Johnston, G. A. R. & Kennedy, S. M. E. in *Amino Acids as Chemical Transmitters* (ed. Fonnum, F.) 507-516 (Plenum, New York, 1978).
- Coyle, J. T. & Schwarcz, R. *Nature* **263**, 244-246 (1976).
- Schwarcz, R. & Coyle, J. T. *Life Sci.* **20**, 431-436 (1977).
- Kurihara, E., Kuriyama, K. & Yoneda, Y. *Expl. Neurol.* **68**, 12-26 (1980).
- Guidotti, A., Toffano, G. & Costa, E. *Nature* **275**, 553-555 (1978).
- Kuriyama, K., Kurihara, E., Ito, Y. & Yoneda, Y. *J. Neurochem.* (in the press).
- Andrews, P. R. & Johnston, G. A. R. *Biochem. Pharmacol.* **28**, 2697-2702 (1979).
- Yoneda, Y. *et al. Jap. J. Pharmacol.* **27**, 881-888 (1977).
- Enna, S. J. & Snyder, S. H. *Molec. Pharmacol.* **13**, 442-453 (1977).
- Nakamura, N. *Chem. Pharm. Bull.* **19**, 46-51 (1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).
- Pellegrino, L. J. & Cushman, A. J. in *A Stereotaxic Atlas of the Rat Brain* (Appleton-Crofts, New York, 1967).

Electrochemical, photoelectrochemical, electrocatalytic and catalytic reduction of redox proteins

A. E. G. Cass, M. J. Eddowes, H. A. O. Hill & K. Uosaki

Department of Inorganic Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, UK

R. C. Hammond, I. J. Higgins & E. Plotkin

Biological Laboratories, University of Kent, Canterbury, UK

Redox proteins catalyse¹ the reactions of a wide variety of otherwise intractable substrates, such as dinitrogen, alkanes, arenes, terpenes and steroids. Two major factors impede the utilization of these enzymes—the inefficient electron transfer between the enzyme and electrode, and the properties often, but not inevitably, associated with enzymes, such as instability, complexity, and expense. We have now shown that the former can be overcome and that proteins can be coupled, via electrodes, to a number of energy sources; the latter is² the subject of much effort elsewhere. We demonstrated previously³⁻⁶ that certain redox proteins can be reduced very efficiently electrochemically (Fig. 1a). Light and hydrogen are the two other convenient energy sources that could be used for such reductions, and we now report the reduction of cytochrome *c* by these means.

When ferricytochrome *c* is reduced to ferrocyclochrome *c* photochemically, an amino acid residue in close proximity of

the haem of the cytochrome is oxidized⁷. This is avoided by using the configuration shown in Fig. 1b, which illustrates photoelectrochemical reduction. The system consisted of TiO_2 (1 cm² polycrystalline)/potassium phosphate buffer (pH 7), 0.1 M NaClO_4 /potassium phosphate buffer (pH 7), 0.1 M NaClO_4 , 10 mM 4,4'-bipyridyl and 100 μM ferricytochrome *c*/Au; it gave an open circuit potential of 350 mV and short circuit current of $\sim 40 \mu\text{A}$ when TiO_2 was illuminated by a 350 W xenon lamp. Water is oxidized to oxygen at a TiO_2 surface⁸ and cytochrome *c* is reduced at the gold surface, the 4,4'-bipyridyl acting³⁻⁶ as a promoter of electron transfer. After 150 min 30% of the cytochrome *c* is reduced as monitored by spectroscopy. Two methods with dihydrogen as a reducing agent were used; electrocatalytic and catalytic reduction. The experimental arrangement for electrocatalytic reduction is shown in Fig. 1c. A Pt-based fuel cell electrode (donated by Johnson & Matthew Ltd) was used as an anode. Other configurations are the same as those used in the photoelectrochemical experiments. When H_2 gas was passed over the fuel cell electrode, an open circuit potential of 600 mV was observed, which is that expected from the relevant redox potentials. A short circuit current of 60 μA was obtained. After 1 h, 30% of cytochrome was reduced. Catalytic reduction was effected as shown in Fig. 1d. Hydrogen gas was passed through 1 ml of potassium phosphate buffer (pH 7) containing 10 mM bipyridyl and 100 μM ferricytochrome *c*. No significant change in the spectrum of the solution was observed until Au gauze (8 cm²) was placed into it. As shown in Fig. 2, the ferricytochrome *c* was reduced, the reduction following first order kinetics with a rate constant of $6.67 \times 10^{-5} \text{ s}^{-1}$.

Although cytochrome *c* was used in these experiments there are many redox proteins which could be reduced by these methods and the possible applications of such redox reactions are wide. For example, the methods could be used in the synthesis of organic compounds. This has already been exploited^{9,10} in electroenzymological syntheses utilizing monooxygenases which catalyse the reaction, $\text{AH} + \text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{AOH} + \text{H}_2\text{O}$, the reducing equivalents being supplied electrochemically. For example camphor can be oxidized to *exo*-5-hydroxycamphor using the configuration shown in Fig. 1a and a wide variety of hydrocarbon substrates can be oxidized electroenzymologically using the methane monooxygenases present in extracts of methanotropic bacteria^{9,10}. When the enzymes concerned can be reduced at relatively positive potentials, one can use light or dihydrogen as a reducing agent and synthesize compounds with electricity as a by-product compare (Fig. 1b and c) or efficiently drive the reaction catalytically (Fig. 1d).

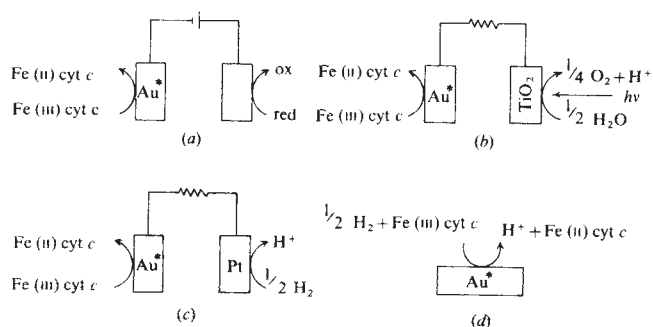


Fig. 1 Reduction of cytochrome *c* by various methods a, Electrochemical: reduction by external source. b, Photoelectrochemical: illumination of TiO_2 excites electron which reduces cytochrome *c* at a gold electrode. c, Electrocatalytic: H_2 gas passed over Pt fuel cell electrode is ionized and supplies electron which reduces cytochrome *c* at a gold electrode. d, Catalytic: H_2 gas is passed over gold at which cytochrome *c* is reduced. In all cases, Au^* is gold modified by 4,4'-bipyridyl.

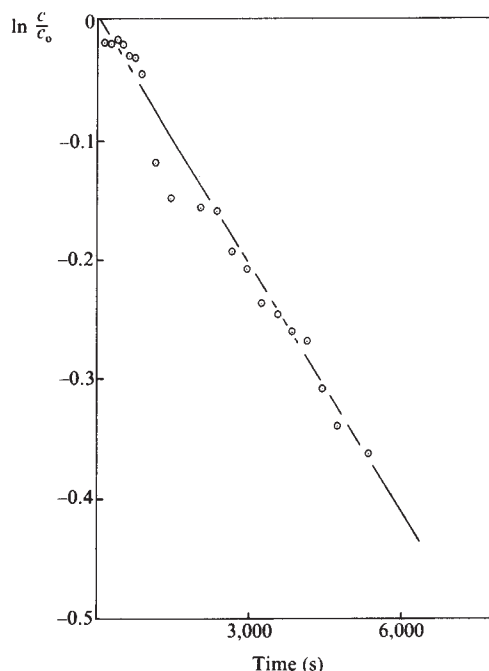
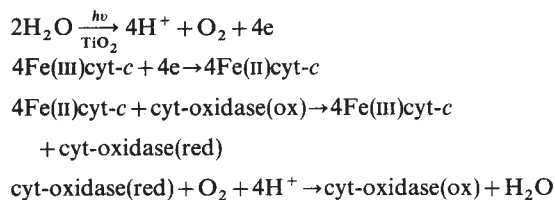
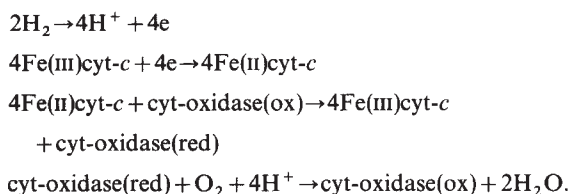


Fig. 2 Time dependence of the concentration of reduced and oxidized form of cytochrome *c* in the case of catalytic reduction of cytochrome *c* by hydrogen on gold. c_0 and c are the concentration of Fe(III)cytochrome *c* at time 0 and at time t .

When the generation of electricity is the main concern, one can apply these systems as photoelectricity generators or bio-fuel cells. For example, the TiO_2 /cytochrome *c*, cytochrome oxidase/Au system will give electricity by illuminating TiO_2 as in the reactions shown below with no net chemical reaction except for the conversion of light to electricity:—



Also the (H_2) Pt/buffer solution//cyt-*c*, cyt-oxidase/Au(O_2) system will give electricity with the net chemical reaction of $2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$. This is a bio-fuel cell resulting from the reactions given below:



In view of the fact that the oxygen reduction reaction has not yet been perfected this latter application could be useful.

We thank the SRC and the National Research Development Corporation for financial support.

Received 31 December 1979; accepted 2 April 1980.

- Boyer, P. D. (ed.) *The Enzymes* Vols XII, XIII (Academic, New York, 1975, 1976).
- Chibate, I. *Immobilized Enzymes; Recent Developments* (Wiley, New York, 1978).
- Eddowes, M. J. & Hill, H. A. O. *Chem. Commun.* 771–772 (1977).
- Eddowes, M. J. & Hill, H. A. O. *J. Am. chem. Soc.* **101**, 4461–4464 (1979).
- Eddowes, M. J., Hill, H. A. O. & Uosaki, K. *J. Am. chem. Soc.* **101**, 7113 (1979).
- Eddowes, M. J., Hill, H. A. O. & Uosaki, K. *Bioelectrochem. Bioenergetics* (in the press).
- Vorkink, W. P. & Cusanovich, M. A. *Photochem. Photobiol.* **19**, 205–215 (1974).
- Honda, K. & Fujishima, J. *Nature* **238**, 37–38 (1972).
- Higgins, I. J. & Hill, H. A. O. U.K. Patent application 33388178.
- Higgins, I. J. *et al.* in *Hydrocarbons in Biotechnology* (Harrison, D. E. F., Higgins, I. J. & Watkinson, R. J.) (Institute of Petroleum, London, in the press).

Isolation and mapping of a cloned ribosomal protein gene of *Drosophila melanogaster*

Charles A. Vaslet, Peter O'Connell, Marta Izquierdo* & Michael Rosbash

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254

Molecular cloning techniques are particularly well suited to the study of gene organization in *Drosophila melanogaster* because recombinant DNA can easily be localized in the genome by *in situ* hybridization to salivary gland polytene chromosomes. We report here the isolation and preliminary characterization of a recombinant phage, designated C25, containing a *bona fide* *D. melanogaster* ribosomal protein gene. *In situ* hybridization demonstrates that this sequence maps to region 99D on chromosome 3.

To isolate segments of *D. melanogaster* DNA containing ribosomal protein (r-protein) genes we purified a probe enriched for r-protein mRNA and hybridized a cDNA copy of it to a *D. melanogaster* genomic library contained in the λ -derived cloning vector Charon 4. Previous work has demonstrated that many yeast r-proteins are encoded by low molecular weight poly(A)⁺ mRNA^{1,2}. Because the number and molecular weight range of r-proteins from yeast³ and *Drosophila*⁴ are similar, we purified a 6S–12S poly(A)⁺ mRNA probe for screening. Electrophoretic analysis of the wheat-germ translation products obtained from this small mRNA revealed that the probe was highly enriched for mRNAs coding for proteins of molecular weight less than 20,000, among which were many ribosomal proteins (data not shown).

The 6S–12S poly(A)⁺ mRNA was labelled to a high specific activity (10^8 c.p.m. per μg) with AMV reverse transcriptase⁵ and used to screen a *D. melanogaster* DNA library in Charon 4 (ref. 6). After plaque purification, candidate phage were analysed for the presence of r-protein genes. We identified these phage by a method devised for the purpose and also used to identify r-protein genes from yeast⁷. Briefly, it consists of incubation of cloned DNA and RNA in the presence of high formamide concentrations to generate R-loops, separation of the R-loops from unhybridized RNA by gel filtration chromatography, and *in vitro* translation of the RNA able to form R-loops. The *in vitro* translation product resulting from one of the first 10 clones examined, designated C25, is shown in Fig. 1b and will be the subject of the remainder of this report. The evidence that this protein is a *D. melanogaster* r-protein is as follows. First, this *in vitro* translation product co-migrates on a two dimensional gel with a known *D. melanogaster* r-protein of approximately 20,000 daltons, designated r-protein 49 (Fig. 1). Second, a peptide map analysis of protein 49 and this *in vitro* product verifies that the C25 gene product is indeed identical to the known 20,000-dalton r-protein. To do this, both the *in vitro* and *in vivo* labelled proteins were purified on two-dimensional gels and subjected to proteolysis with *Staphylococcus aureus* V8 protease⁸. The digests produced by this enzyme were composed of several peptide fragments whose molecular weights were sufficiently different to facilitate their separation on an SDS slab gel; the two proteins gave identical peptide patterns in a side-by-side comparison (Fig. 2). These results are consistent with the notion that C25 contains a DNA sequence homologous to an mRNA which directs the synthesis of a *D. melanogaster* ribosomal protein.

*Present address: Centro de Biología Molecular, Universidad Autónoma de Madrid, Canto Blanco, Madrid 34.

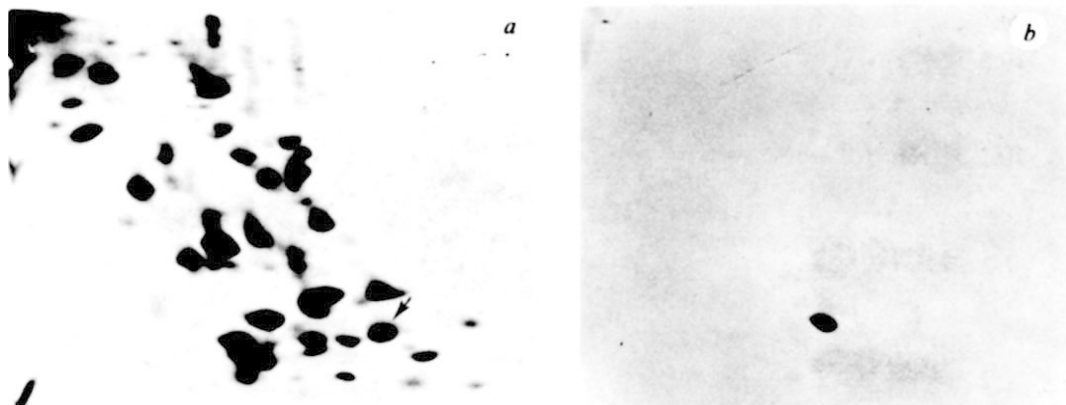


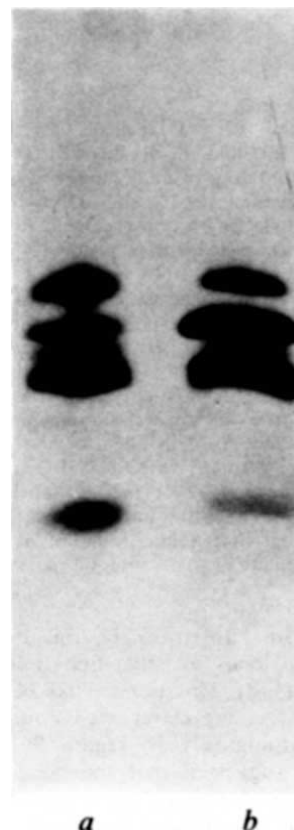
Fig. 1 Two-dimensional fluorogram of total *D. melanogaster* ribosomal proteins labelled *in vivo* with ^{35}S -methionine (a) and clone C25 gene product similarly labelled *in vitro* (b). An arrow indicates r-protein 49 encoded by C25. Two-dimensional gel electrophoresis was done by the method of Mets and Bogorad¹⁹. After electrophoresis, gels were subjected to fluorography according to Bonner and Laskey²⁰. The procedure for labelling and purifying r-proteins was as reported by Berger²¹. C25 gene product was obtained by cell-free translation of mRNA complementary to phage DNA as reported by Woolford *et al.*⁷. Poly(A)⁺ containing 3S–14S mRNA prepared as before²² was subfractionated on a 15–30% continuous sucrose density gradient made up in SDS buffer (0.5% SDS, 0.1 M NaCl, 0.01 M Tris, pH 7.5, 0.001 M EDTA) in order to isolate the 6S–12S material used in screening the *Drosophila* library. All other polysomal RNA was prepared by a modification of the method of Lis *et al.*²³. Embryos 6–18 h old were homogenized at 4°C in a buffer containing 0.25 M KCl, 0.025 M MgCl₂, 0.05 M Tris, pH 7.4, 0.5% NP-40 and cycloheximide (200 $\mu\text{g ml}^{-1}$). The homogenate was centrifuged in a Sorvall SS34 rotor at 17,000 r.p.m. for 30 min at 4°C to remove debris. Polysomes were collected as a pellet prepared by centrifuging the low speed supernatant through a 10-ml cushion of 50% sucrose in homogenization buffer lacking NP-40 and cycloheximide for 8 h at 25,000 r.p.m. in a Beckman SW25.2 rotor at 4°C. The polysomal pellet was resuspended in 1% SDS, 50 mM Tris, pH 9, 1 mM EDTA, 15 mM Na acetate and was extracted 3 times with phenol–chloroform–isoamyl alcohol. The RNA was pelleted and dissolved in 0.1 M Tris, pH 8.3, 0.03 M EDTA, 0.1 M NaCl, 1% SDS containing proteinase K (300 $\mu\text{g ml}^{-1}$). After a 30-min incubation at 37°C, the preparation was extracted with phenol as above and the RNA was alcohol-precipitated. Poly(A)⁺ mRNA was obtained by hybridization to oligo(dT)-cellulose columns²⁴.

Fig. 2 Fluorogram of peptide mapping by limited proteolysis with *S. aureus* V8 enzyme⁶. The patterns represent only ^{35}S -methionine-labelled peptides from digests of clone C25 gene product (a), and the known 20,000 dalton r-protein with which it co-migrates on a two-dimensional gel (b). Proteins were isolated by punching out spots from preparative two-dimensional gels run as described previously. Care was taken to insure that each plug cut from the gel contained only one electrophoretic mobility class. Protein isolates were digested without prior elution by placing the gel plugs in the sample wells of a second SDS gel containing 20% acrylamide and overlaying with 10 μg *S. aureus* V8 protease in 10 μl of 0.125 M Tris-HCl, pH 6.8, 0.17% SDS, 1 mM EDTA and 10% glycerol. Electrophoresis was performed as usual, except that the current was turned off for 30 min when the bromphenol blue tracking dye neared the bottom of the stacking gel.

To determine the size of the *Drosophila* DNA insert in clone C25 an *EcoRI* restriction digest was performed and the fragments were separated on an agarose gel. In addition to the 19.5- and 10.2-kilobase arms of the Charon vector, fragments of 7.2, 5.5, 2.6 and 2.4 kilobases indicated a total insert size of 17.7 kilobases (Fig. 3). These fragments were ordered by subjecting C25 DNA to single and double digestion with the restriction enzymes *EcoRI*, *XbaI* and *HindIII*. The arrangement of these fragments is supported by an analysis of three C25 *HindIII* fragments subcloned into the plasmid vector pBR322 (data not shown).

To investigate the arrangement of this 17.7 kilobase segment of cloned DNA within the *Drosophila* genome, hybridizations to *Drosophila* genomic DNA were carried out with labelled C25 as a probe⁹ (Fig. 3). The bands detected in the genomic DNA lane are identical to C25 DNA with respect to molecular weight and similar with respect to intensity. This suggests that the 17.7 kilobase insert which includes an r-protein structural gene is therefore largely, if not entirely, single copy. The number and location of the coding regions present in this sequence remain to be defined although several distinct R-loops have been visualized in preliminary electron microscopic studies (unpublished observations).

Having determined that clone C25 contains a structural gene for a *D. melanogaster* r-protein, we mapped its genomic position by *in situ* hybridization^{10,11}. An ^3H -nick translated¹²



probe was prepared from total C25 DNA and hybridized to squashes of salivary gland polytene chromosomes. As Fig. 4 shows, labelled DNA from C25 hybridized exclusively to chromosome 3R at region 99D. This result was found in several preparations and in no instance were grains observed over any other chromosome arm. This location is interesting because some data had suggested the X-chromosome linkage of r-protein genes in *D. melanogaster*^{13,14}. Although our findings do not refute this possibility for some ribosomal proteins, they demonstrate that at least one r-protein structural

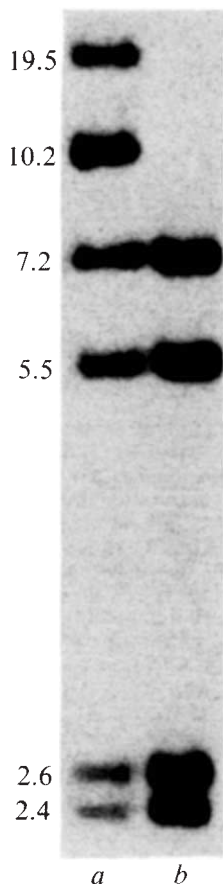


Fig. 3 Autoradiograph of ^{32}P -labelled C25 hybridized to *Eco*RI-digested C25 (a), and *Eco*RI-digested *D. melanogaster* genomic DNA (b). In a 0.6 ng of *Eco*RI-digested C25 was mixed with 2 μg of *Eco*RI-digested yeast carrier DNA to approximate the mass of a C25 single length copy sequence within 2.0 μg of the 165,000-kilobase *D. melanogaster* genome. In b 2.0 μg of *Eco*RI-digested *D. melanogaster* DNA was loaded. These samples were electrophoresed on 1% agarose gels. The 19.5- and 10.2-kilobase fragments seen in a correspond to the Charon 4 vector DNA. High molecular weight *D. melanogaster* DNA was prepared from 6–18-h old embryos (a gift of S. Elgin) as described by Schachat and Hogness²⁵. The DNAs were denatured and transferred to nitrocellulose paper in $10\times\text{SSC}$. Hybridizations were carried out in 50% formamide, $5\times\text{SSC}$, $1\times\text{Denhardt's}$ solution, 20 mM NaPO_4 , and sonicated salmon sperm DNA ($100\mu\text{g ml}^{-1}$) at 42°C . *In vitro* labelled C25 DNA was prepared utilizing the nick-translation technique described by Maniatis *et al.*¹², except that a small amount of DNase I was added to stimulate incorporation of nucleotides.

gene is on an autosome. Interestingly, this map position is close to the genetic locus of the first minute mutation discovered, *M(3)l* (ref. 15). This minute has been mapped 0.3 map units to the right of the claret eye-coloured gene which has been mapped cytologically to region 99 C-E (ref. 16). Ritossa *et al.*¹⁷ have suggested that the positions of minute loci represent tRNA genes although this hypothesis seems no longer viable¹⁸. Their prediction had been based on the similarity in number of minute loci and tRNAs and the phenotypic abnormalities associated with the minutes (bristle deformities, delayed development and small size) suggesting a reduction in general protein synthesis. We are intrigued by the possibility that the 60 or more minute mutations may be ribosomal protein mutants. This possibility and some of its implications are under investigation.

We thank J. Woolford, U. Schafer, L. Hereford, L. Golden, J. Hall and K. Fahrner for helpful discussions, and members of N. Davidson's laboratory, in particular K. Soratkin, for access to and help in screening their *D. melanogaster* genomic

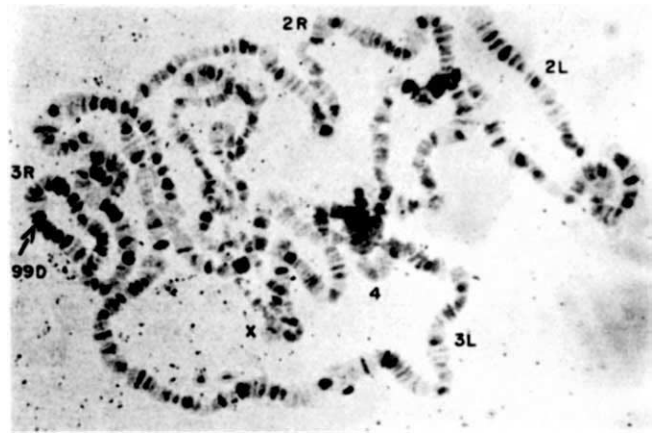


Fig. 4 Salivary gland chromosome squashes were prepared for hybridization by a modification of the procedures of Atherton and Gall¹⁰ and Pardue and Gall¹¹. Before hybridization slides were treated with pancreatic RNase at $1\mu\text{g ml}^{-1}$ in $2\times\text{SSC}$ for 2 h at 37°C . The RNase was washed from the slides with several rinses in $2\times\text{SSC}$ for 10 min, followed by an overnight wash with gentle swirling in $2\times\text{SSC}$ containing 1 mM EDTA. The slides were then dehydrated through a series of ethanol baths (70%, 70% 95% and 95%) and air dried. Chromosomal DNA was denatured in 0.07 M NaOH for 1.5 min at room temperature and then the slides were again passed through an ethanol series and air dried. *In situ* hybridization of ^3H -labelled nick-translated clone DNA was done under 22-mm² coverslips in a total incubation volume of 20–30 μl (approximately 2×10^5 c.p.m. per slide and 10^6 c.p.m. per μg DNA). The hybridization buffer contained $2\times\text{SSC}$, 2.5 mM Tris, pH 7.5, and 50% formamide. Reactions were carried out at 37°C for 16 h in a buffer-saturated chamber. After hybridization, the slides were washed in hybridization buffer at 37°C for 60 min, then in several changes of $2\times\text{SSC}$ for 3 h at room temperature. The slides were finally dehydrated through an ethanol series, air dried, dipped in Kodak NTB-2 liquid emulsion and stored at 4°C for 2 weeks. The slides were developed for 2.5 min in Kodak D-19 and the chromosomes were stained with Giemsa stain diluted 1:20 in 0.01 M NaPO_4 buffer, pH 7.0. The chromosome position of high grain densities was determined by using Bridge's polytene chromosome maps²⁶ as interpreted by Lefevre²⁷. The arrow points to grains found over region 99D of chromosome 3.

library. C.V. was supported by a postdoctoral fellowship from the American Cancer Society. M.R. is a recipient of a Research Career Development Award from NIH. This study was supported by grants HD08887 and GM23549 from NIH.

Received 4 December 1979; accepted 16 April 1980.

1. Mager, W. H. & Planta, R. *Eur. J. Biochem.* **62**, 193–197 (1976).
2. Warner, J. R. & Gorenstein, C. *Meth. Cell Biol.* **20**, 45–60 (1979).
3. Otaka, E. & Kobata, K. *Molec. gen. Genet.* **162**, 259–268 (1978).
4. Berger, E. *Molec. gen. Genet.* **128**, 1–9 (1974).
5. Hereford, L. M. & Rosbash, M. *Cell* **10**, 453–462 (1977).
6. Benton, W. D. & Davis, R. W. *Science* **196**, 180–182 (1977).
7. Woolford, J., Hereford, L. & Rosbash, M. *Cell* **18**, 1247–1259 (1979).
8. Cleveland, D. W. *et al. J. biol. Chem.* **252**, 1102–1106 (1977).
9. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
10. Atherton, D. & Gall, J. *Drosoph. Inf. Serv.* **49**, 131–133 (1972).
11. Pardue, M. L. & Gall, J. *Meth. Cell Biol.* **10**, 1–16 (1975).
12. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184–1188 (1975).
13. Steffensen, D. M. *Nature new Biol.* **244**, 231–234 (1973).
14. Falke, E. V. & Wright, T. R. *Genetics* **81**, 655–682 (1975).
15. Lindsley, D. L. & Grell, E. H. in *Genetic Variations of Drosophila melanogaster* (Carnegie Institution of Washington, 1971).
16. Bridges, C. B. & Morgan, T. H. in *The Third Chromosome Group of Mutant Characters of Drosophila melanogaster* (Carnegie Institution of Washington, 1923).
17. Ritossa, F. M., Atwood, K. C. & Spiegelman, S. *Genetics* **54**, 663–676 (1966).
18. Elder, R., Szabo, P. & Uhlenbeck, O. in *Transfer RNA: Biological Aspect* (Cold Spring Harbor Laboratory, New York, 1980).
19. Mets, L. J. & Bogorad, L. *Analyt. Biochem.* **57**, 200–210 (1974).
20. Bonner, W. H. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
21. Berger, E. *Molec. gen. Genet.* **155**, 35–40 (1977).
22. Izquierdo, M. & Bishop, J. O. *Biochem. Genet.* **17**, 473–497 (1979).
23. Lis, J. T., Prestidge, L. & Hogness, D. S. *Cell* **14**, 901–919 (1978).
24. Rosbash, M., Ford, P. J. & Bishop, J. O. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3746–3750 (1974).
25. Schachat, F. S. & Hogness, D. S. *Cold Spring Harb. Symp. quant. Biol.* **38**, 371–381 (1974).
26. Bridges, C. P. J. *Hered.* **26**, 60–64 (1935).
27. Lefevre, G. in *The Genetics and Biology of Drosophila 1a* (eds Ashburner, M. & Novitski, E.) (Academic, New York, 1976).

BOOK REVIEWS

Hearing voices: the public planning process after Windscale

Frederick Warner

THESE books have a common origin in the Windscale Public Inquiry (WPI). That from the Town and Country Planning Association in association with the Political Ecology Research Group summarizes evidence given and usefully supplements the bibliography in Vol. 3 of the Windscale Report. The other is a report by Professor D. Pearce on a project for which he received a grant from the Social Science Research Council Energy Panel, of which he is a member. It expresses his concern at the way in which opposition groups were treated and on the efficiency of public planning inquiries in general and Windscale in particular.

Professor Pearce heads a department of political economy and his book emphasizes the political nature of the planning process. He uses the Windscale Inquiry as an illustration of one element in a system for planning which he sees as dominated by official thinking and insufficiently open. This hampers objectors who have insufficient access to information and lack the resources to prepare for the compressed time-scale of a public inquiry. The system also prevents the questioning of values — for example that the development would be unnecessary with a simpler life-style or with a stricter examination of needs. It also obscures substantial disagreement on the risks involved and the benefits to be obtained.

The remedy, according to Professor Pearce, lies in new procedures and institutions which will become more efficient through a Freedom of Information Act: "The public can also improve decision-making by raising issues that decision-makers may have overlooked or underplayed". They would be helped in this by an Energy Policy Commission reviewing decisions on investment and R&D programmes. The Commission would monitor local planning applications and, where wider issues were involved, recommend review by a Planning Inquiry Commission (PIC), reporting to Parliament through a Select Committee; it would provide funds for objectors to make representations to the PIC and the local

The Nuclear Controversy: A Guide to the Issues of the Windscale Inquiry. By M. Stott and P. Taylor. Pp.204. (Town and Country Planning Association: London, 1980.) £6.95. *Decision Making for Energy Futures.* By D. Pearce, L. Edwards and G. Beuret. Pp.296. (Macmillan: London and Basingstoke, 1979.) £10.

planning inquiry; it would be full-time and high-powered, in contrast with the existing Commission on Energy and the Environment chaired by Lord Flowers.

These criticisms are said to arise in part from the time-table for the Windscale Inquiry (shown on p.135 of the book) as announced on 22 December 1976, opening on 14 June, closing on 4 November 1977, and reporting on 26 January 1978. However, Professor Pearce's discussion of the issues begins in 1974 when the plan for re-processing oxide fuel was announced, and carries through the Church House debate in January 1976 between opposition groups and British Nuclear Fuels Ltd to the Flowers Report of the Royal Commission in September 1976. Given the scope of the Flowers Report it is not easy to see how the debate was hampered for lack of information and would have been bettered by an Energy Policy Commission.

There is a difficulty in assessing Professor Pearce's argument. He disclaims particular intentions and then reinstates them: "Criticism of a process is not therefore a criticism of the individuals who find themselves part of that process. Clearly, a disagreement can exist if they defend that process as Mr Justice Parker has done publicly" (p.132). In reviewing these books, I have to face this possibility, but more readily as the independence of the Inquiry has in any case been questioned.

Professor Pearce is critical of the lack of examination at WPI of economics or the engineering aspects of scaling-up. The evidence on costs was made difficult because the commercial secrecy of the contract with the Japanese, with its payments in advance, led to erroneous discounted cash flow forecasts by the

objectors. The evidence on the need for a pilot plant reflected old-fashioned chemical engineering practice and was irrelevant to the actual scale of the Windscale operations which are more the size of pilot plants themselves.

He begins with the effects of low-level radiation and on p.146 states that "The history seems important because, whatever the format of an inquiry, personalities intervene and what Professor Radford, and other witnesses, did was to question the very role of ICRP and the validity of its work as a basis for setting standards. Yet one of the assessors, Sir Edward Pochin, was, and is, a prominent member of ICRP". ICRP is the International Commission on Radiological Protection and Professor Radford chairman of the US National Academy of Sciences Committee on Biological Effects of Ionizing Radiation (BEIR). This is due to publish a report which will show Professor Radford in disagreement with his committee. One member, Dr Fabrikant, in a March 1980 paper (Jerusalem) says: "... such disagreement centres not on the scientific facts or the epidemiological data, but rather on the assumptions and interpretations of the available facts and data. However, based on the radiation risk estimates derived ... such risks are extremely small when compared with those available from alternative options".

The ICRP publications, in particular 26 and 27, give guidance for the comparison of risks and some economists have attempted to make comparisons, the most recent being Siddall in Canada (Chalk River, 12–14 September, 1979) with CSX, the cost of saving one life. Professor Pearce has lost time between writing and publication so that his study suffers from being overtaken by events. This is shown by the references to the International Fuel Cycle Evaluation Programme (INFCEP) announced in April 1977 by President Carter. Its report in March 1980 has now cleared up many of the questions. It states that proliferation cannot be prevented by technical fixes such as spiking nuclear fuel, that there is an urgent need to meet world

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Mr. Justice Parker (centre) who headed the Windscale enquiry, with Sir Frederick Warner (left) and Sir Edward Pochin.

energy requirements with nuclear power, especially for developing countries, and that some countries need to build and operate fast reactors and hence to re-process nuclear fuel. Professor Pearce criticizes Mr Justice Parker for not referring in the Windscale Report to the letter to the Foreign Office from Joseph Nye, Presidential Adviser, dated 19 December 1977, yet makes clear on p.90 that the Report could only refer to evidence

given to the Inquiry.

The march of time is also shown by the section on energy futures. This compares the scenario of the Department of Energy in 1977 with those put forward by Leach and Chapman during WPI. Since then the 1979 figures of the Department, which show reduced estimates of supply and demand, have been put to the Belvoir Inquiry. The nuclear component is only slightly less than the 1977 estimate, but coal

comes down from the 170 million tons for the year 2000 to 135, and oil from 150 to 100 Mtce. The Leach demand analysis was discussed in the last report of the Advisory Council for Energy Conservation with the conclusion that with his assumptions the figures are not in basic disagreement with the Department's. Professor Pearce does not accept that official views need be given greater credence than unofficial and (p.155) criticizes "references to some of the forecasts (that of Arthur Scargill of the Yorkshire Area Mineworkers Union) as 'fanciful'. It is presumably not possible to know what is 'fanciful' without having a prior view of what is reasonable". This is the place to use the TCPA/PERG guide (p.57) "Silsoe questioned Scargill's figures for new investment required, and the rate of new pit openings in order to achieve this projected target of 250 million tons by 1992".

Camera Press

Throughout Professor Pearce's book, the Windscale Inquiry receives grudging tributes for doing the job at all, in contrast with the regard in other countries, especially France, for the openness and seriousness of its attempts to hear every voice. It achieved in fact what his new bureaucratic apparatus (p.205) could never do in providing information and guidance which is sought by the public. □

Sir Frederick Warner is a Consulting Chemical Engineer and Visiting Professor at University College, University of London, UK.

Reactors from Russia

Jeffery Lewins

Fast Pulsed and Burst Reactors. By E.P. Shabalin. Pp.260. (Pergamon: Oxford, 1979.) £26, \$59.

THIS monograph is translated from the Russian, by W.E. Jones, in the Pergamon tradition of scientific translation. And a good job he has made of it. Generally the translation reads very well with occasional confusion between Russian letters and Roman or Greek letters.

Russian translations do have a minor defect in regard to the double translation of English or American names quoted in references to Russian and back again. Keepin for example will be interested to read of his sea change to Kipin and Paul Gribler for Greebler is in for a similar surprise. More seriously — and a defect avoidable in these days of automated library science — the return translation does not always identify the text the original was (in many cases) pirated from before Russia joined the copyright protocol. I speak as an author pirated without knowing, let alone agreeing, and as an editor of a series left here with only its Russian edition for reference.

But to the book. It is a detailed exposition of the physics of fast reactors used in pulse and burst mode, that is applied as dynamic research tools. Additional material is given on certain engineering aspects such as thermal elastic effects, dynamic control etc., as well as to combined boosters (neutron sources and sub-critical multipliers). The text is completed with a chapter on the peculiar aspects of safety and a look ahead to current and future applications of these devices for physics research.

The text reads well. The first five chapters were, it seemed to me, reasonably clear of misprints but the standard (in a readable typescript production) fell off in Chapters 6 to 10, however. Most of the errors I caught were trivial. Occasionally there were muddles between the el and one so tedious to get correct on a typewriter (for example eqn 6.12), and also eqn 6.3 would benefit from a couple of extra primes.

The book is a valuable summary of the special theory of dynamic fast reactors and a comparative source of their leading characteristics, both Eastern and Western, which I have not met elsewhere. □

Jeffery Lewins is a Lecturer in Nuclear Engineering at the University of Cambridge, UK, and is a Past President of the Institution of Nuclear Engineers.

Gel chromatography

Torvard C. Laurent

Gel Chromatography: Theory, Methodology and Applications. By T. Kremmer and L. Boross. Pp.300. (Wiley: Chichester, UK, and New York, 1979.) £16.50, \$45.

ALTHOUGH earlier reports appeared on the separation of substances according to molecular size when chromatographed on granulated gels, the introduction of gel chromatography as a preparative and analytical method can be dated to 1959, to a paper by Porath and Flodin in *Nature* (1959, 1657-1659). The following decade saw a rapid development of the practical and theoretical aspects of the technique, to a large extent due to the collaborative investigations of scientists at the University of Uppsala and Pharmacia Fine Chemicals. During the last 20 years, gel chromatography has become one of the major tools in many fields of research and manufacturing. Many names have been used for the procedure (gel filtration, exclusion chromatography, molecular sieving, gel permeation chromatography etc.) but gel chromatography has now been adopted by most reviewers of the field.

Several books or articles have been

written about the technique, and this volume, by Tibor Kremmer and László Boross, is the most recent. It has been translated from an original Hungarian version and contains three main chapters: "Theory", "Methods and Techniques", and "Applications". The first two chapters were written by Kremmer and the last by Boross. The authors have cited a large amount of the earlier work in the field but their description is not very lucid and contains numerous errors. Furthermore, a

number of ambiguities have been introduced in the translation. The chapter on methods and techniques relies heavily on literature produced by the manufacturers of gels and chromatographic equipment; manufacturers' handbooks do, however, usually present the material in a clearer fashion.

The value of the book could have been that of a reference volume on the applications of gel chromatography in various fields; unfortunately, however, it is

not up to date. The last reference cited is from 1976 and of the references only one out of six is from the five-year period 1972–76.

Given the lack of clarity and the dated section of references, this book is not an important addition to the literature on gel chromatography. □

Torvard C. Laurent is Professor of Medical and Physiological Chemistry at the University of Uppsala, Sweden.

Authority and politics on iron metabolism

William H. Crosby

Iron Metabolism in Man. By T. H. Bothwell, R. W. Charlton, J. D. Cook and C. A. Finch. Pp. 576. (Blackwell Scientific: Oxford, 1980.) £33.50.

THIS superb review and analysis is the product of four scientists, two South African, two American, whose long careers have been engrossed with iron nutrition and iron metabolism. It is a comfortably written book, authoritative and completely documented (more than 2,600 references) up to 1978. It is a book that I shall use and treasure for the rest of my life.

The organization of the material departs from the ordinary in that a 240-page section on nutritional and clinical aspects comes first, followed by discussions of the basic physiology of iron metabolism. A third section (100 pages) describes in detail the laboratory methods that are used to study iron metabolism. Think of some aspect of iron in man and — except for 'spleen' — you'll find it listed in the index and discussed in the text in satisfactory detail.

All this is not to imply that there's no worm in the bud. During the past 20 years, the authors have been activists in the politics of nutrition, committed to universal fortification of foods with iron. Such programmes are intended to prevent mild iron deficiency. (It has yet to be demonstrated that fortification improves mildly iron-deficient people or injures those with undiagnosed iron-storage diseases, such as haemochromatosis.) The book is marked by a bias that favours fortification even at the expense of objectivity. Mild iron deficiency is treated with a concern worthy of a dread disease while the potentially lethal haemochromatosis is brushed across because it is rare, as though the improvement of a laboratory result in many could offset deadly damage done to only a few.

However rare haemochromatosis is, it

would seem to be not rare enough. In a discussion of the evidence that haemochromatosis may develop only in homozygotes comes this observation: "Sheinberg has estimated the incidence of homozygous births as not more than 1 in 10,000 and heterozygous 1 in 50; the latter figure in particular seems at this time to be much too high". That the latter figure derives from the other is a matter of genetics and arithmetic, not opinion.

At one place in the book, the authors compute that a woman with normal iron stores would, during a normal pregnancy, have a requirement for an additional 740mg of iron. In another place, it is recommended that such women should receive 30mg per day of "supplemental

iron". In yet a third place, we find this polemic: "The presence of medicinal iron in the homes of pregnant women is responsible for the deaths of small children from acute iron poisoning, and nutritional deficiency must be held responsible for this too".

Nutritional anaemia, in this setting, refers to the iron deficiency that results from copious menstrual blood loss.

There are other statements as unfortunate as these. Yet, in balance, the book is the best of its class; that it is also a vehicle for propaganda is sad. □

William H. Crosby is at the Walter Reed Army Institute of Research, Washington DC.

Periglacial processes six years on

R. J. E. Brown

Geocryology: A Survey of Periglacial Processes and Environments. By A. L. Washburn. Pp. 416. (Edward Arnold: London/Halsted Press: New York, 1979.) £27.50, \$64.95.

INCREASING recognition of the importance of resource developments in the cold areas of the Earth has focused continuing and growing concern on environmental conditions and physical processes in these regions. In 1973, A. L. Washburn published *Periglacial Processes and Environments* (Edward Arnold: London), which brought together the knowledge and ideas on this topic to that time. The present book is a revised and updated edition of that volume.

The author is one of the world's foremost authorities on processes taking place in cold regions, with more than 30 years' experience of the subject. He states that his book "... is intended as a rather comprehensive overview of periglacial processes and their effects, present and past ... The book is neither a formal text nor a reference manual but something of both and a guide to the enormous

literature. Geocryology is included in the title to emphasize the pervasive influence of ice and its phase changes in these processes. ... The term is in fact used in the restricted sense as applying to frozen ground (seasonally frozen ground as well as permafrost) but not to glaciers."

The 1973 edition was an impressive and most useful text; this volume is equally so, being monumental in its coverage of the field. No one involved in periglacial investigations can afford to be without it. The format of the new book with hard cover, 7½" × 10" page size and a full page of text in two columns is an improvement on the earlier work with its horizontal 8" × 10" page size, smaller amount of text per page and single columns. The tremendous volume of literature that has appeared since 1973 and the author's thorough treatment of it are demonstrated by a comparison of the two editions. The 1973 edition has 262 pages, the 1979 publication 320 pages. However, the total text of the latter is nearly twice that of the former. The larger print of the earlier work is slightly easier to read but the smaller typeface used in the new work is no drawback. One of the most impressive aspects, and certainly an indication of the phenomenal increase of literature over six years, is the current bibliography. From about 1,000 references in 1973, the list has grown to about 2,700. This is reflected in the text by the presence of one or more citations on virtually every line. I experienced some difficulty at first in

Environmental Chemistry

**R. W. Raiswell,
P. Brimblecombe, D. L. Dent
and P. S. Liss**

*Resource and Environmental
Sciences Series*

A general introduction to the chemical concepts which are relevant to environmental topics.

Paper £4.95 192 pages

Publication July

Environmental Toxicology

John H. Duffus

*Resource and Environmental
Sciences Series*

An interdisciplinary approach to the analysis of environmental effects of toxic substances.

Paper £4.95 144 pages

Publication August

First Year Chemistry

**J. M. Coxon, J. E. Fergusson,
L. F. Phillips**

An introductory chemistry text for undergraduates which aims at a balanced, unified approach with inorganic, organic and physical chemistry being given equal weight and emphasis being placed on the concepts which they share.

Boards £9.75 384 pages

Elementary General Relativity

C. Clarke

An ideal introduction to general relativity, special relativity, gravitation and weak-field theory. Full mathematical proofs are given where necessary which will be useful to readers requiring a more detailed knowledge of the subject.

Paper £5.95 144 pages

A Modern Course in Statistical Physics

L. E. Reichl

This text provides a comprehensive study of all the new techniques and developments in statistical physics. It is an ideal reference for students and researchers.

*Boards £20 approx 700 pages approx
Publication July*



Edward Arnold
41 Bedford Square,
London WC1B 3DQ

literally wading through a sea of parentheses, but one quickly becomes accustomed to it. This encyclopaedic format is in fact very successful in quickly acquainting the reader with the literature. The index items have been increased in number from about 800 to 1,000. There are also more illustrations, about 200 instead of 150. Finally, the tables number 23 as compared with 10 in the earlier version. It will be interesting to see how much more periglacial literature is produced by 1985.

For such an impressive publication I have only a few criticisms, generally of a minor nature. The title of the earlier work seems more suitable to the contents of both books. The inclusion of "Geocryology" does not seem necessary especially in the more restricted sense specified by the author. Soviet permafrost workers use the term to refer to the study of the frozen zones of the Earth's crust. The author restricts its use to emphasizing the pervasive influence of ice in frozen ground. Somehow it seems redundant to include it in the title. "Periglacial" says it all because it includes frozen ground together with all its associated features.

The material on palsas and peat plateaux is somewhat thin, but I should admit to a bias because of my own particular interest in such landforms. There is now a large body of literature on investigations into the origin and development of these

controversial features. Some photographs and diagrams would have suitably filled out this section. Russian terms for palsas are confusing. Actually the Russian word 'bugor' means only 'mound' and does not refer specifically to palsas. They could use the term 'palsa' but do not choose to do so. The closest approximation is 'torfyanii bugor' or 'peat mound'. The Russian (Yakutian) word 'bulgunnyakh' actually is used for 'pingo' or 'hydrolaccolith' and is not used to refer to palsas.

Chapters 7 to 12 inclusive are each very short and produce an imbalance in the text. Perhaps some sort of grouping of these short chapters might have been possible. Chapters 6, 7 and 8 could have possibly been integrated into a single section.

Finally, it is regrettable that the new book is so expensive, putting it completely out of the reach of students. Nevertheless, every library should have at least one copy. Serious research workers of the periglacial will welcome it as a prime source of information.

The painstaking effort that has gone into this work is truly remarkable and a great tribute to the experience of the author. I very much hope that he will be able to do a repeat performance in 1985 — he is certainly the one for this immense task. □

R. J. E. Brown is a Senior Research Officer at the National Research Council of Canada, Ottawa, Ontario.

Island botany

F.R. Fosberg

Plants and Islands. Edited by D. Bramwell. Pp.442. (Academic: London and New York, 1979.) £24, \$55.50.

THIS SUBSTANTIAL volume comprises the papers given at an international symposium on plants and islands, held in April 1977 in Las Palmas, Canary Islands.

The papers cover a wide spectrum of knowledge of island botany, both in subject matter and geography, though mention of the vast island areas of Polynesia and Micronesia is conspicuously absent from the list of titles. Tahiti and Fiji are discussed only incidentally, the Marquesas as the home of remarkable endemics, and Hawaii as an example of the results of destruction of flora and vegetation by human agency. Understandably, five of the chapters are specifically on Macronesian floras.

The contribution are arranged in four sections, titled "Origins", "Endemism and Evolution", "Special Topics" and "Conservation".

The papers, generally, are interesting, mostly well prepared and give, for the subjects covered, a summary of the present state of knowledge and of the unfortunate present condition of the floras of most

islands. Much of our knowledge must be pieced together from accounts of nineteenth-century visitors, fragmentary herbarium records and relicts of the vegetation as it was when European visitors first saw the islands.

The fascination that islands and their floras hold for so many biologists is well conveyed by most of the chapters. Effective dispelling of some of the exaggeration of the peculiarities of island floras is to be found in the provocative papers by Mabberley and Guédès. A masterly overview, such as we have come to expect from Ehrendorfer, is presented on reproductive biology in island plants and on several related topics. This chapter should be read, particularly by those who are concerned with the difficult problem of the preservation of the numerous endangered species on islands. Endemism is treated intensively in three chapters and less so in most others, but some of the discussions are marred by the use of largely undefined polysyllabic terminology.

Ideas involving plate tectonics and continental drift pervade many of the chapters, and provide explanations for many otherwise intractable problems. These ideas, long propounded by some biologists, have now become respectable and accepted by the formerly sceptical geological profession. However, one sometimes has the feeling that the time-frame in which the crustal movements took

place seems to stretch unduly the age of some of the Angiosperm taxa concerned.

It is a pity that the discussions of these papers that must have taken place were not recorded and published in appropriate places in the volume. Some fascinating remarks must have been exchanged.

Mabberley's concluding paragraph (p.274) points out one of the handicaps of researching into plant geography: the piecemeal approach fostered by the circumstances of modern research support and the difficulty of undertaking long-term work on large taxonomic groups. The poor understanding that flaws our knowledge of large and complicated genera is reflected in unsound plant geographic hypotheses. "Who has time to use his intellect to unravel the stories of *Cassia*, *Eugenia*,

Euphorbia, *Piper*, *Solanum* or *Vernonia* and their pachycauls?" Corner, in a secure position without too great a burden of teaching, was able to point the way in his studies of *Ficus*.

The editing is generally good, though a few errors slipped by such as the indication in Table 1 (p.173), of a total native flora of 65 species for the Cape Verde Islands. Several of the diagrams are rather hard to follow. The quality of reproduction of the half-tones (e.g. Figs 1-4, pp. 264-265) leaves much to be desired.

One gets the impression that the selection of topics, or perhaps the selection of speakers, was somewhat hit or miss, and that the only basic organization was that the papers must be on plants and islands. Here and there, there seems to be some

relation between the topics, but the section on conservation is the only one that tends to come into focus as a whole, and even there it seems that perhaps each author did not know that the others were writing. However, the conservation prospects on islands are so uniformly dismal that perhaps a consistent impression in this section was unavoidable.

The price of the book will keep it out of reach for most of those who will want it. However, it is a boon to all students of island floras and biogeography that this symposium was published and is available.

F. R. Fosberg is a Botanist Emeritus at the Smithsonian Institution, Washington DC, with an interest in the geography and ecology of island floras.

The great British drought

Timothy O'Riordan

Atlas of Drought in Britain 1975-76. Edited by J.C. Doornkamp, K.J. Gregory and A.S. Burn. Pp.82. (Institute of British Geographers: London, 1980.) £27.50.

FOR A natural hazard that has no recorded parallel since at least 1698, and may have a probability of occurrence of 0.001, the great British drought of 1975-76 left remarkably little impact. For most of the public it has become lost in faded memory, while groundwater supplies, surface water resources, agricultural production and even most nature reserves are restored to normality. Yet the event was an important one in many ways, so this impressive production by the British Geomorphological Research Group in collaboration with the talented cartographic unit at the University of Southampton provides a valuable service.

The objective of the Research Group was to present a pictorial image of both the physical and the economic-administrative aspects of a major natural hazard. This is a worthy motive, but it necessarily involves a lot of collaboration amongst people who are already geared up to study the event and, if all the relevant researchers are not available at the right time, the result can be patchy and incomplete. The first section on the climatological characteristics of the drought is well done with excellent analyses of rainfall, sunshine and evaporation statistics for Britain and continental Europe. The blocking anticyclone that was responsible for the drought of 1976 was really the Mediterranean high pressure transferred some 1,000 km to the north — a shift probably caused by an unusually cold North Pacific which in turn moved the North Atlantic jet stream well to the north. As a result some depressions moved into the Mediterranean where, over the 18-month period, higher than average

rainfalls were recorded. Surprisingly in this section there is no record of temperature.

The second section covers the hydrological impacts of the drought in

Britain. The national reviews of rainfall, evaporation, groundwater levels and soil moisture are well documented, but the rest of this section is rather uneven for the editors were dependent on workers who happened to be in the field at the time, writing up their case studies. The result is an interesting set of cameos but little in the way of a national perspective.

In economic terms the drought seems to have been remarkably friendly. The editors wisely avoid calculating a specific figure, but apart from a 40% loss of root and horticultural crops in the south-east, much of British agriculture was not savagely affected and apparently recovered quickly in the heavy rains that followed. The insurance companies certainly paid out a lot on claims for property damage due to clay shrinkage but will doubtless have raised their premiums since. The water authorities are now wiser about how far the public can be persuaded to cut consumption (up to 25% in the worst-hit areas following a sustained campaign) and are armed with much more specific powers to withhold water from non-essential customers (mainly domestic properties) during times of drought.

The large page format (30mm × 42mm), while making the book a little unwieldy, does mean that many diagrams can be presented on one page and that the text of each contribution can be fitted in without requiring the turning of a page. The diagrams are beautifully drawn in a uniform style. A lot of work has gone into this production and the editors are to be congratulated for their efforts. But the task of recording the varied dimensions of such an event is a formidable one, and even this stalwart effort falls a little short of what would be desirable. It is a particular pity that the scale and nature of recovery were not also recorded for this would have helped greatly to place the drought in proper perspective. □

Timothy O'Riordan is Professor of Environmental Sciences at the University of East Anglia, Norwich, UK.

The reservoir at Pitsford, 5th May 1976, 20 ft below normal for the time of year.

Keystone Press

BOOKS RECEIVED

General

- CURRAN, S. C. and CURRAN, J. S. *Energy and Human Needs*. Pp. xix + 330. ISBN-0-7073-0237-4. (Edinburgh: Scottish Academic Press, 1979.) £8.50.
- GRIFFIN, J. *Future Worlds*. Pp. 225. London: Sphere Books Limited, 1979.)
- FAST, Herausgegeben von G. Orchideen kultur. *Botanische Grundlagen, Pflanzenbeschreibungen*. Pp. 460.) DM 98.
- FEUERSTEIN, R. *et al.* *Instrumental Enrichment. An Intervent Program for Cognitive Modifiability*. Pp. xxiii + 436. ISBN-0-8391-1 (Lancaster: MTP Press, Ltd. 1980.) £12.95.
- FRITZ, C. *Combating Nutritional Blindness in Children. A Case Study of Technical Assistance in Indonesia*. Pergamon Policy Studies-57. Pp. x + 205. ISBN-0-08-024636-2. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon, 1980.) £7.50.
- FREUDENSTEIN, R. (ed.) *Teaching Foreign Languages to the very young. Papers from seven countries on work with 4-to-8-year olds*. Pp. xii + 97. ISBN-0-08-024576-5. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) £2.95 \$5.95.
- GARFIELD, E. (ed.) *Transliterated Dictionary of the Russian Language. An abridged dictionary consisting of Russian-to-English and English-to-Russian sections*. Pp. xi + 382. ISBN-0-89494-003-7 hardback ISBN-0-8945-011-8 flexi. (Philadelphia: ISI, 1980.) \$25.00 hardback \$14.95 flexi.
- GIBSON, W. B. *SRI the Founding Years. A significant step at the golden time*. Pp. x + 212. ISBN-0-913232-80-7. (California: William Kaufmann, Inc., 1980.) \$20.50 (domestic) \$25.00 (foreign).
- GOLDSTEIN-JACKSON, K. *Activities with Everyday Objects*. Pp. ISBN-0-285-62439-3. (London: Souvenir Press, 1980.) £4.95.
- GORDON, M. J., MILNER, A. J. and WADSWORTH, C. P. *Edinburgh LCF. A Mechanised Logic of Computation. Lecture Notes in Computer Science*. 78. Pp. viii + 159. ISBN-3-540-09724-4. (Berlin, Heidelberg, New York: Springer-Verlag, 1979.) DM 21.50 approx. US \$12.10 flexi.
- HARTLEY, W. C. F. *An Introduction to Business Accounting for Managers*. Third edition. Pergamon International Library of Science, Technology, Engineering and Social Studies. Pp. xiv + 221. ISBN-0-08-024062-3 flexi ISBN-0-08-024061-5 hardback. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon, 1980.) \$11.25 £4.95 flexi \$24.00 £10.50 hardback.
- HEATHCOTE, R. L. and THOM, B. G. (ed.) *Natural Hazards in Australia. Proceedings of a Symposium sponsored by Australian Academy of Science, Institute of Australian Geographers, Academy of the Social Sciences in Australia*. Pp. xii + 531. ISBN-0-85847-056-X. (Canberra: Australian Academy of Science, 1979.) A\$20.00.
- INMAN, W. H. W. (ed.) *Monitoring for Drug Safety*. Pp. xxv + 673. ISBN-0-85200-234-3. (London: MTP Press, Limited, 1980.) £24.95 \$24.95.
- I.R.S.I.A. *Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel 1978*. Pp. 343. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture.) flexi.
- JOYNER, N. T. Jr. *Joyner's Guide to Official Washington for Doing Business Overseas. Pergamon Policy Studies on U.S. and International Business*. Pp. xxi + 364. ISBN-0-08-025108-0. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon, 1980.) £47.50 \$95.00.

Psychology

- FORGUS, Ronald, and SHULMAN, Bernard. *Personality: a Cognitive View*. Pp. xiii + 434. ISBN-0-13-657882-9. (Englewood Cliffs, N.J., and Hemel Hempstead: Prentice-Hall, Inc., 1979.) £11.
- GROTH, A.N. *Men Who Rape: The Psychology of the Offender*. Pp. xviii + 227. ISBN-0-306-40268-8. (New York and London: Plenum, 1979.) \$15.00.
- GURKAYNAK, M.R. and LeCOMPTE, W.A. (ed.) *Human Consequences of Crowding*. Pp. ix + 331. ISBN-0-306-40298-X. (New York and London: Plenum Press, 1979.) \$32.50.
- HAINES, Richard F. (ed.) *UFO Phenomena and the Behavioral Scientist*. Pp. xiv + 450. ISBN-0-8108-1228-2. (Metuchen, N.J. Scarecrow, Inc., 1979.) \$18.50.
- HARDY, Alistair. *The Spiritual Nature of Man*. Pp. viii + 162. ISBN-0-19-824618-3. (Oxford: Oxford University Press, 1979.) £6.95.
- IBRAHIM, M.A. *The Case-Control Study Consensus and Controversy*. Pp. iv + 144. ISBN-0-08-024907-8. (Oxford, New York, Paris, Frankfurt: Pergamon Press, 1979.) \$25.00, £12.50.
- LEWIS, M. and BROOKS-GUNN, J. *Social Cognition and the Acquisition of Self*. Pp. xix + 296. ISBN-0-306-40232-7. (New York and London: Plenum, 1979.) \$19.50.
- SMILANSKY, M. and NEVO, D. *The Gifted Disadvantaged; A Ten Year Longitudinal Study of Compensatory Education in Israel*. Pp. x + 245. ISBN-0-677-04400-0. (New York, London, Paris: Gordon & Breach, 1979.) £12.50.

Applied Biology

- McNAMARA, J.R. (ed.) *Behavioural Approaches to Medicine. Application and Analysis*. Pp. xxiii + 311. ISBN-0-306-40238-6. (New York and London: Plenum Press, 1979.) \$25.00.
- MEJIA, A., PIZURKI, H. ROYSTON, E. (reported by.) *Physician and Nurse Migration. Analysis and Policy Implications. Report of a WHO Study*. Pp. xiv + 476. ISBN-92-4-156059-2. (Geneva: World Health Organisation, 1979.) Sfr.64.
- MILAN, F. A. (ed.) *The Human Biology of circumpolar populations. International Biological Programme 21*. Pp. xv + 381. ISBN-0-521-22213-3. (Cambridge, London, New York, New Rochelle, Melbourne, Sydney: Cambridge University Press, 1980.) £35.00.
- NETH, R., GALLO, R.C. HOFSCHEIDER, P.-H. and MANNWEILER, K. (ed.) *Modern Trends in Human Leukemia III. Newest Results in Clinical and Biological Research. 9th Scientific Meeting of "Gesellschaft Deutscher Naturforscher und Ärzte"*, June 19-23, 1978. Pp. xxi + 599. ISBN-3-540-08999-3. (Berlin, Heidelberg, New York: Springer, 1979.) Flexi.
- PHILLIPS, G.O. TALLENTIRE, A. and TRIANTAFYLLOU, N. (ed.) *Radiation Sterilization: Irradiated Tissues and their potential Clinical use. Papers presented at an Advisory Group Meeting*. Pp. x + 242. (Clwyd: The North Wales Institute, 1978.)
- SUTHERLAND, I. (ed.) *Health Education: Perspectives and Choices*. Pp. xi + 273. ISBN-0-04-371069-7. (Hemel Hempstead: George Allen & Unwin, 1979.) £10.00.
- WORLD HEALTH ORGANIZATION. *Specifications for Pesticides used in Public Health. Insecticides, Molluscicides, Repellents, Methods*. Fifth Edition. Pp. 327. ISBN-92-415410-7. (Geneva: World Health Organization, 1979.) SFR. 48.00.
- WORLD HEALTH ORGANIZATION. *The International Pharmacopoeia Third edition. Volume 2. General Methods of Analysis*. Pp. 223. ISBN-92-4-154150-4. (Geneva: World Health Organization, 1979.) SFR. 24.00.

THE STRUCTURE OF ELEMENTARY PARTICLES AND GRAVITATION

AS SEEN BY AN ORGANIC CHEMIST

Z. J. ALLAN

Suppose that elementary particles are built of the well known electrons and neutrinos rather than of the yet undetected quarks and that their properties depend on the number and the ratio of the included leptons. Such particles can be arranged in a periodical table like the elements. The calculated binding energies explain the stability of the proton. All symmetries, including that of the parity, become unviolable again.

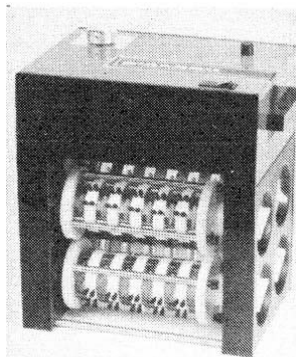
The electrons are woven from photons. Thus all forces have an electromagnetic basis and a common field. Even the gravitational force can be calculated from the qualities of the electron. The cosmological deductions give simple models of black holes, the big bang and the huge cosmical energies.

PUBLISHED 1980, PAPER COVERS, ILLUSTRATED, £2.90 or SF 11.50. PLEASE ORDER FROM:

Z. J. ALLAN,
Bruggartenweg 10,
CH-4123 Allschwil,
SWITZERLAND.

DIANORM® Equilibrium Dialyzing System

To quantify the binding parameters and thermodynamic data of reactions between ligands and biopolymers (K_{ass} , K_{diss} , n , N , ΔG , ΔH , ΔS). The conventional and time-honoured 'knotted bag' technique belongs to the past. DIANORM brings scientific discipline, precision and speed.



Up to 20 leak-free cells from PTFE (half-cell volumes 0.2ml, 1.0 ml, 2.0 ml or 5.0 ml) that are easy to fill and empty reproducibly. Membrane area is standardized, optimal concentration gradients across the membrane are obtained. For control of temperature the cells can be submerged in a water bath. Minimum degradation of labile samples.

- Precision — up to one order of magnitude improvement in comparison with the 'knotted bag' technique.
- Speed — about 30 minutes to reach equilibrium for ligands of low molecular weight.
- Convenience — 20 experiments can be set up in less than 30 minutes.

Today DIANORM® is in routine use for drug binding studies well known all over the world and part of the standard equipment of research laboratories in

**Biophysics — Pharmacology — Pharmacokinetics —
Biochemistry — Molecular Biology — Clinical Chemistry —
Nuclear Medicine — Food Chemistry.**